European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

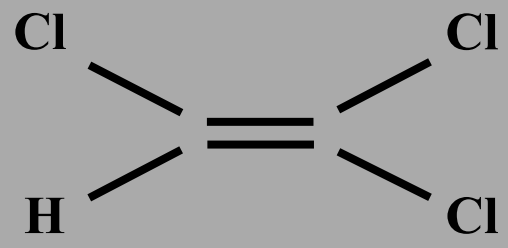
Existing Substances

European Union Risk Assessment Report

CAS No: 79-01-6

EINECS No: 201-167-4

trichloroethylene



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PL-1

31

1st Priority List

Volume: 31

EUROPEAN COMMISSION
DIRECTORATE-GENERAL
Joint Research Centre

EUR 21057 EN

European Union Risk Assessment Report

TRICHLOROETHYLENE

CAS No: 79-01-6

EINECS No: 201-167-4

RISK ASSESSMENT

Printed in Italy

TRICHLOROETHYLENE

CAS No: 79-06-1

EINECS No: 201-167-4

RISK ASSESSMENT

Final Report, 2004

United Kingdom

This document has been prepared by the UK rapporteur on behalf of the European Union. The scientific work on the environmental part was prepared by the Building Research Establishment Ltd (BRE), under contract to the rapporteur.

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Date of Last Literature Search:	**1995**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2004**

(The last full literature survey was carried out in 1995 - targeted searches were carried out subsequently: DCA-related search 1998; air levels search 2000).

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.

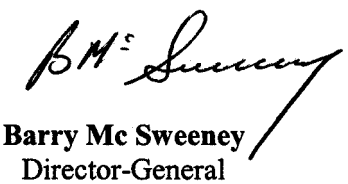
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each 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, which 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.



Barry Mc Sweeney
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Director-General
DG Environment

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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.: 79-01-6
EINECS-No.: 201-167-4
IUPAC name: Trichloroethylene

Environment

This assessment does not address risks arising from groundwater contamination.

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

This conclusion applies to the risk of harm to plants from air emissions of trichloroethylene from production, processing as an intermediate, formulation for solvent use, and use in metal degreasing. The Solvent Emissions Directive (1999/13/EC) will have an impact on the emissions of this substance, in particular on use in metal degreasing. The conclusion for production applies to two sites. The conclusion for processing as an intermediate applies to sites which did not provide emission information.

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 applies to the aquatic compartment (including sediment), to wastewater treatment plants, to the terrestrial environment and to secondary poisoning for all stages in the production and use of trichloroethylene; to the atmospheric compartment for adhesive formulation and use, consumer product formulation and use, and "other" uses; and to the aquatic and terrestrial compartments for dichloroacetic acid produced by the photodegradation of trichloroethylene.

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.

This conclusion is reached for all scenarios for mutagenicity and carcinogenicity, because it is not possible to identify a threshold exposure level below which these effects would not be expressed and therefore there are concerns for human health at all exposures. There is no evidence that the controls currently in place across all industry sectors in the EU represent best practice for a mutagenic and carcinogenic substance, therefore conclusion (iii) is reached for all exposure scenarios. For all scenarios, there are concerns for repeated dose kidney toxicity and conclusion (iii) applies. There are concerns for acute CNS depression for metal cleaning, adhesive manufacture (without LEV) and adhesive use and conclusion (iii) applies. There are concerns for repeat dose functional CNS disturbance for metal cleaning, adhesive manufacture (irrespective of LEV use) and adhesive use and conclusion (iii) applies.

It is not possible to draw clear conclusions regarding developmental neurotoxicity. Further testing according to OECD TG 426 is needed. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen, the results of such testing are unlikely to influence the outcome of the risk assessment, as the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

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

For all uses, the risk of skin and eye irritation is considered to be low, providing good occupational hygiene practices are in operation, and conclusion (ii) applies.

Consumers

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

The substance gives rise to concerns for humans owing to possible mutagenic and carcinogenic effects, because it is not possible to identify a threshold exposure level below which these effects would not be expressed. In view of the potential for consumer exposure, there are concerns for human health as a result of the consumer use of trichloroethylene and conclusion (iii) applies.

It is not possible to draw clear conclusions regarding developmental neurotoxicity. Further testing according to OECD TG 426 is needed. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen, the results of such testing are unlikely to influence the outcome of the risk assessment, as the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

Humans exposed via the environment

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

For carcinogenicity and mutagenicity endpoints, there is no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels. However, the predicted regional and environmental exposures are very low. Therefore, although there may be some residual risk of mutagenicity and/or carcinogenicity this is likely to be very low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

It is not possible to draw clear conclusions regarding developmental neurotoxicity. Further testing according to OECD TG 426 is needed. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen, the results of such testing are unlikely to influence the outcome of the risk assessment, as the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

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

In relation to acute CNS effects, skin and eye irritation, repeated dose functional CNS disturbance and repeated dose kidney toxicity, there is no significant risk for humans exposed via environmental routes.

Combined exposure

The potential combined exposure is dominated by the occupational exposure. Thus, the conclusions of the risk characterisation for combined exposure reflect those reached for workers.

Human health (risks from physico-chemical properties)

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

If the appropriate conditions of handling and storage are adhered to, there are no concerns for risks to human health arising from the physicochemical properties of trichloroethylene.

CONTENTS

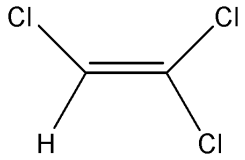
Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTITY OF THE SUBSTANCE

CAS-No.:	79-01-6
EINECS-No.:	201-167-4
IUPAC name:	Trichloroethylene
Synonyms:	Acetylene trichloride, Ethinyl trichloride, Trichloroethene, TRI, TRIC, 1-Chloro-2,2-dichloroethylene, 1,1,2-Trichloroethylene, Trilene, Triklone®
Molecular weight:	131.39 -131.5 (131.5 used in this assessment)
Molecular formula:	$C_6 H_3 Cl_3$
Structural formula:	



1.2 PURITY / IMPURITIES, ADDITIVES

1.2.1 Purity

The purities quoted in IUCLID were all $\geq$ 99.9 % w/w.

The significant impurities (where stated) comprised some or all of the following (expressed as % w/w):

Tetrachloroethylene	<0.03%
Vinylidene chloride	<0.01%
1, 1, 1-Trichloroethane	<0.01%
Chloroform	<0.01%
Carbon tetrachloride	<0.005%
Dichloromethane	<0.001%
Bromodichloromethane	< 0.1%
Water	trace

The impurities present vary according to the plant and production method.

1.2.2 Additives

The stated additives present in trichloroethylene available from various suppliers included the following (% w/w):

Thymol	$\leq$ 1%
Triethylamine	$\leq$ 1%
Trimethyloxirane	$\leq$ 0.45%

Ethyl acetate	≤ 0.7%
2, 4, 4-Trimethylpentene	0.25 - 0.3%
Butanone	0.22 - 0.22%
Epoxybutane	0.22 - 0.3%
1-Methylpyrrole	0.02 - 0.022
Diisopropylamine	< 0.005%
2 -Methyl-3 butan-2-ol	< 4%
2, 4-Di-tertbutylphenol	<50 ppm
1, 2-Butylene oxide	<0.6%
Glycidyl ether	< 0.8%

These compounds are added as stabilisers.

1.3 PHYSICO-CHEMICAL PROPERTIES

1.3.1 Physical state (at ntp)

Trichloroethylene is a colourless non flammable liquid with a characteristic odour resembling that of chloroform. The odour is detectable at around 20 to 30 ppm.

1.3.2 Melting point

The melting point of trichloroethylene has been reported as -84.7 (CRC Handbook, 1994) to -87°C (Verschueren, 1983). A value of -84.8 is reported in the consolidated IUCLID data set; the same value is reported in Lange's Handbook (1992) and the Merck Index (11th Edition, 1989). A value of -85°C is reported in NFPA (1994) and of -86.5°C in Kirk-Othmer (4th Edition, 1991). The value of -84.8 will be taken as the melting point.

1.3.3 Boiling point

The boiling point of trichloroethylene has been reported as 85.9 to 88 °C. A value of 85.9°C is reported by Korte and Greim (1981). The consolidated IUCLID entry is 86 to 87°C, Lange's Handbook (1992) and the Merck Index (1989) report 86.7°C, NFPA (1994), the CRC Handbook (1994), Kirk-Othmer (1991) and Beilstein report values of 87, 87.2, 87.3 and 88°C, respectively. These values are consistent with what would be expected from vapour pressure studies; the value to be used for the purposes of this assessment is between 86 and 88°C.

1.3.4 Density

The bulk density (at 20°C) of trichloroethylene is reported as 1.458 to 1.478 $g.cm^{-3}$. The consolidated IUCLID entry and the Merck Index (1989) report 1.465 $g.cm^{-3}$, Lange's Handbook (1992) and Kirk-Othmer (1991) 1.478 $g.cm^{-3}$, NFPA (1994) 1.46 $g.cm^{-3}$, and the CRC Handbook (1994) presents a range of 1.458 to 1.464 $g.cm^{-3}$. The value of 1.465 $g.cm^{-3}$ reported in the IUCLID data set will be used in this assessment.

1.3.5 Vapour pressure

The vapour pressure of trichloroethylene has been reported in the consolidated IUCLID entry as 86 hPa at 20°C, 90.8 hPa at 25°C and 590 hPa at 70°C. The value of 86 hPa is also reported in Verschueren (1983). An additional value of 78.7 hPa at 20°C is reported by Korte and Greim (1981). McDonald (1944) and Hertz and Rathmann (1912) have carried out vapour pressure studies and this information is presented in Appendix A. The IUCLID and literature data are consistent; a vapour pressure of 86 hPa at 20°C will be used for modelling purposes.

1.3.6 Solubility

Trichloroethylene is practically insoluble in water according to the Merck Index (1989), slightly soluble according to the CRC Handbook (1994) and soluble ($1.1\ g \cdot l^{-1}$) according to the consolidated IUCLID entry (from Horvath, 1982). Lange`s Handbook (1992) reports a solubility of 0.1 % in water and Kirk-Othmer (1991) reports a value of 0.107 % ($1.07\ g \cdot l^{-1}$). A value of $2.85\ g \cdot l^{-1}$ is reported by Korte and Greim (1981). The descriptions of "soluble" and "practically insoluble" are clearly not exact but trichloroethylene has a limited solubility in water at approximately the $1\ g \cdot l^{-1}$ level. The solubility of $1.1\ g \cdot l^{-1}$ will be used for modelling purposes.

Trichloroethylene is miscible with ethanol, ether and chloroform. It is soluble in acetone.

1.3.7 Partition coefficient

A log K_{ow} of 2.6 for trichloroethylene is reported in The Official Journal of the European Communities (1992). It is not clear how this value was determined. The consolidated IUCLID data set presents values of 2.29 (measured) and 2.42 (calculated); the former value is reported in Rogers and McFarlane (1981), the latter in Hansch and Leo (1979, 1985). Further values of 2.42 and 2.98 are reported in Banerjee et al. (1980) and Korte and Greim (1981) respectively. The measured value of 2.29 will be used for modelling purposes.

A sediment-water partition coefficient of 2.1 has been calculated by the US EPA (1982).

1.3.8 Flash point

No flash point was presented in the consolidated IUCLID data set. Trichloroethylene does not have a flash point or fire point according to the chemical handbooks.

1.3.9 Autoflammability

A value of 410°C is given in the consolidated IUCLID data set (Solvay, 1993). No value is given in the Merck Index (1989) or the CRC Handbook (1994). Lange's Handbook (1992) suggests an autoflammability temperature of 420°C, a value also quoted by NFPA (1994).

Trichloroethylene has a flammable range when high concentrations are mixed with air and exposed to high energy ignition sources (Scott, 1963; NFPA, 1994). NFPA suggest flammable limits in air of 8 - 10.5% at 25°C. Kirk-Othmer (1991) quoting from Scott (1963) gives a range of 12.5 - 90% at 25°C. Lange's Handbook quotes 8.0% - saturation. The flammable limit depends upon the circumstances - the ignition source, the energy of the ignition source and the situation in which the vapour is present.

Trichloroethylene would not be classified as "flammable" and it is unlikely that trichloroethylene would be flammable except in unusual circumstances - perhaps where vapour is contained in a sealed vessel and exposed to high energy ignition sources.

1.3.10 Explosivity

According to the consolidated IUCLID data set, trichloroethylene is not explosive. However, violent decomposition is possible under certain conditions in the presence of aluminium (Metz and Roedig, 1949; Archer and Simpson, 1977). Commercial grades of trichloroethylene have stabilisers added to prevent such reactions in normal use and storage.

1.3.11 Oxidising properties

According to the consolidated IUCLID data set, trichloroethylene does not have oxidising properties. This is consistent with the structure.

1.3.12 Surface tension

A value of 0.0293 $N \cdot m^{-1}$ has been reported (Atochem IUCLID, 1995).

1.3.13 Other physico-chemical properties

A vapour density of 0.42 $kg.m^{-3}$ (air =1) is reported in the Merck Index (1989). A Henry's law constant (H) of $1.03 \cdot 10^{-2}$ $atmosphere \cdot m^3 \cdot mole^{-1}$ has also been reported (Atochem IUCLID, 1995). The Henry's Law constant calculated from the ratio of vapour pressure to solubility is $1.03 \cdot 10^3$ $Pa \cdot m^3 \cdot mole^{-1}$, and this value has been used in the EUSES calculations.

A conversion factor of 1 ppm to 5.47 $mg \cdot m^{-3}$ (at 25°C 760 mm Hg) has been used. An alternative value of 5.46 is reported by Verschueren (1983).

1.3.14 Summary of physico-chemical properties

A range of information on physicochemical properties has been reported on trichloroethylene. For several of the properties, particularly water solubility, n-octanol-water partition coefficient and vapour pressure, the range is quite broad and there are one or two outlying values. While the values reported by some of the handbooks may simply be the repeated quoting of one value from earlier sources, these values have been taken as the best measurements for these properties and have been used subsequently for environmental modelling.

The physicochemical properties of trichloroethylene are summarised in **Table 1.1**.

Table 1.1 Summary of physico-chemical properties of trichloroethylene

Properties	Value
Molecular weight	131.5
Melting Point	-84.8°C
Boiling Point	86-8°C
Density	1.465 $g \cdot cm^{-3}$
Vapour pressure	86 hPa at 20°C
Water solubility	1,100 $mg \cdot l^{-1}$
Log octanol/water partition coefficient	2.29
Log sediment/water partition coefficient	2.1 (calculated)
Flammability	lower limit 12.5%, upper limit 90%
Autoflammability	410°C
Vapour density	0.42 $kg \cdot m^{-3}$ (air = 1)
Henry's law constant	$1.03 \cdot 10^{-2}$ $atm \cdot m^3 \cdot mole^{-1}$
Surface tension	0.0293 $N \cdot m^{-1}$ at 20°C
Conversion factor	1 ppm = 5.47 $mg \cdot m^{-3}$

1.4 CLASSIFICATION

Classification and labelling according to the 28th ATP of Directive 67/548/EEC[4]:

Classification

Carc. Cat.2; R45	May cause cancer
Muta. Cat. 3; R68	Possible risk for irreversible effects
Xi; R36/38	Also irritating to eyes and skin
R67	Vapours may cause drowsiness and dizziness
R52-53	Harmful to aquatic organisms; May cause long-term adverse effects in the aquatic environment

Specific concentration limits: None
Note: 6

Carcinogen Category 2 is for substances which should be regarded as if they are carcinogenic to humans. There is sufficient evidence to provide a strong presumption that human exposure to a substance may result in the development, of cancer, generally on the basis of:

1. appropriate long-term animal studies;
2. other relevant information.

[4] The classification of the substance is established by Commission Directive 2001/59/EC of 6 August 2001 adapting to the technical progress for the 28th 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 L 225, 21.8.2001, p.1).

Mutagen Category 3 is for substances which cause concern for humans owing to possible mutagenic effects. There is evidence from appropriate mutagenicity studies, but this is insufficient to place the substance in Category 2.

In addition, Note 6 applies to the labelling of preparations that contain trichloroethylene; such preparations have to be assigned R67 if they meet the appropriate criteria.

Labelling

T	
R: 45-36/38-52/53-67	
S: 53-45-61	Avoid exposure – Obtain special instructions before use In case of accident or if you feel unwell seek medical advice immediately (show the label where possible) Avoid release to the environment. Refer to special instructions/safety data sheet

2 GENERAL INFORMATION ON EXPOSURE

Information in this section on quantities produced and used in the EU relates to the period 1993-1996. Some changes from these figures may have occurred in subsequent years, but these are not expected to affect the outcome of the assessment significantly.

2.1 PRODUCTION

According to the IUCLID data provided, European production of trichloroethylene is between 51,000 and 225,000 tonnes/annum. However, more recent information from the European Chlorinated Solvents Association (ECSA) indicates that production of trichloroethylene in the European Union (EU) was 138,000 tonnes per annum in 1996. Of this, 77,000 tonnes were sold into the EU for uses other than as a chemical intermediate (see below). The remaining 61,000 tonnes were either exported or used as an intermediate, but no breakdown between export and intermediate use is available for 1996. More recent information (personal communication, ECSA, 2001) indicates a similar level of sales within the EU to that for 1996, with use as an intermediate of ~45,000 tonnes. These values are used in the estimates of emissions below.

There are four companies producing trichloroethylene in the EU. Production at a typical plant ranges from 1,000 to 50,000 tonnes/annum. However, there are also a number of agents acting as distributors for smaller amounts of imported trichloroethylene. Information on the balance between production and imports is not available. A few companies also recycle a relatively small amount of trichloroethylene.

In the EU the use of trichloroethylene has declined by over 50% since the mid 1970s. This decline is a result of improved operating conditions leading to better solvent recovery and the use of other chlorinated solvents for metal cleaning.

2.1.1 Production methods

Most of the trichloroethylene produced is derived from ethylene or 1,2-dichloroethene (ethylene dichloride) by chlorinating or oxychlorinating using various catalysts (Nielsen and Howe, 1992).

Ethylene dichloride, produced by the chlorination of ethylene, may be further chlorinated to trichloroethylene at 280-450°C using catalysts such as potassium chloride, aluminium chloride, Fuller's earth, graphite, activated carbon or activated charcoal.

Trichloroethylene can be prepared via oxychlorination of ethylene dichloride in a pressurised reaction at temperatures of about 425°C, using catalysts such as mixtures of potassium and copper chlorides. Oxychlorination is used to produce tetrachloroethylene and trichloroethylene at the same facility (crude production figures may therefore include both chemicals). The resulting trichloroethylene/tetrachloroethylene mixture is separated by distillation.

Trichloroethylene and tetrachloroethylene are also produced from the oxychlorination of recovered residues derived from vinyl chloride monomer (VCM) manufacture.

Trichloroethylene is also produced by the catalytic hydrogenation of tetrachloroethene (BUA, 1994). A specially activated copper-palladium catalyst on a carrier material is used in the gas phase at temperatures up to 250°C.

2.2 USES

The major use of trichloroethylene is for vapour degreasing and cleaning of metal parts (WHO, 1985). It is also used in adhesives, for synthesis in the chemical industry and as a solvent for various products, including insecticides and waxes (WHO, 1985). It is (or has been) used in the leather and textile processing industries and in the paint, lacquers and varnishes industry.

According to industry sources, of the trichloroethylene sold, 82% is used for metal degreasing, 9% in adhesives, 6% is for consumer uses and 3% is for other uses (extraction, leather preparation, pharmaceuticals etc.). The figure for consumer use covers domestic textile "spot" cleaning, largely in France, Belgium and Italy. These figures are presented in **Table 2.1**.

Table 2.1 Uses of trichloroethylene sold into the EU market

Use	Percentage of total sales	Quantity used (tonnes/year)
Metal degreasing in vapour degreasers	82	63,140
Adhesives	9	6,930
Consumer uses	6	4,620
Others	3	2,310

Note that some 61,000 tonnes is exported or used as a feedstock chemical intermediate. This tonnage is not included in the above figures.

2.2.1 Metal cleaning

The principal use of trichloroethylene is for metal cleaning. Although information is not available by country for the rest of Europe, in the UK in 1993 approximately 23,000 tonnes were supplied to the metal cleaning industry. The amount used in the UK for degreasing is now understood to be lower than this. It is principally used for hot vapour degreasing of metal components. This is only one of many methods employed to clean metal. Alternatives include other hydrocarbon solvents, aqueous formulations and water blasting or soft blasting (shot blasting using a relatively soft medium such as limestone).

Suppliers do not recommend trichloroethylene for cold cleaning. Continuing improvements in solvent recovery technology on hot vapour degreasing baths has reduced the amount of trichloroethylene used for hot vapour degreasing. These reductions are as a result of better working practices and the use of newer technology. Some companies are also attempting to find non-chlorinated hydrocarbon or water based cleaning agents as alternatives. This is particularly true of the microelectronics industry where the use of trichloroethylene is continually diminishing and will probably disappear altogether.

2.2.2 Adhesives

Trichloroethylene is used in adhesives for which a solvent of low flammability is required that also possesses the desired drying time. Trichloroethylene based adhesives have no specialised application and therefore can be found in any industry. Therefore it has not been possible to profile the use of trichloroethylene in adhesives used in the EU. The use could include any application where a low flammability solvent is required, although for any of these applications alternatives are also likely to be available. An accurate picture of its use in adhesives would only be possible by contacting all adhesive manufacturers using this solvent. This was further

complicated as it was not possible to identify adhesive manufacturers using trichloroethylene. Trichloroethylene can also be supplied as a cleaner for use with the adhesive. Although detailed information is not available for the rest of Europe, in 1993 about 1,000 tonnes were used in the UK in adhesives by an estimated 10 to 15 adhesive manufacturers.

One EU member state has reported a minor use of trichloroethylene during the vulcanisation of rubber conveyor belts in open cast mines. However, many mines use conveyor belts made from solid woven PVC or solid woven PVC with a rubber coating. This is particularly the case in the UK where conveyor belts for use in mines have to meet British Standard (BS3289). It is understood that rubber conveyor belts are used in German open cast mines and that trichloroethylene is used as an adhesive in the vulcanisation process. This is understood to be a minor and infrequent use of trichloroethylene.

2.2.3 Use as an intermediate

Based on information from ECSA, 45,000 tonnes of trichloroethylene is used as a chemical feedstock.

Trichloroethylene is used in the production of HFC 134a (1,1,1,2-tetrafluoroethane) and HCFC 133a (1-chloro-2,2,2-trifluoroethane). HFC 134a is used as a refrigerant and HCFC 133a is used to manufacture the anaesthetic, halothane. In the UK, the volume of trichloroethylene used in the manufacture of HFC 134a and HCFC 133a together is understood to be less than 10,000 tonnes per annum.

It is anticipated that there will be an increase in the demand for trichloroethylene as a feed stock for the manufacture of HFC 134a due to its use as an alternative refrigerant to CFCs, some of which are being phased out under the Montreal Protocol. The figure of 45,000 tonnes takes this into account as it relates to 2000.

Trichloroethylene is also used in the production of pentachloroethane (WHO, 1985). No further information is available on this use.

Trichloroethylene is apparently no longer used as a chain transfer agent in the production of polyvinyl chloride.

2.2.4 Other uses

A significant tonnage of trichloroethylene is ascribed to direct use by consumers. The only consumer use identified is for spot cleaning of fabrics to remove stains. It is also reported to have been used as a food extractant in Spain, but no details have been obtained for this application.

The advent of triacetate fibres in the 1950s led to the replacement of trichloroethylene with tetrachloroethylene in dry cleaning machines, as the latter has a milder solvent action. Therefore there is either little or no use of trichloroethylene as a dry cleaning solvent in the EU.

Trichloroethylene is understood to be used (or to have been used) as a solvent carrier for bitumen coatings, in solvent based phosphating systems, for textile desizing scouring, in leather preparation, the pharmaceutical industry and as a heat transfer agent. Trichloroethylene has also been used as a carrier solvent for pesticides, printing inks, varnishes and paints, as an anaesthetic, and as a solvent for waxes, fats, resins and oils. The information obtained suggests

that it is no longer used for these applications but the extent to which it is so used within the EU has not been established.

2.3 EXPOSURE TO TRICHLOROETHYLENE FROM PRODUCTION AND USE

2.3.1 Environmental releases and exposure

Releases of trichloroethylene may be from point or diffuse sources. Point source releases of trichloroethylene may occur from production and handling of trichloroethylene and through its use as a chemical intermediate. Other releases of trichloroethylene will be from its use as a metal degreasing agent, in adhesives and as a solvent. These releases are likely to occur from many different sites and so this is a diffuse release on a regional scale. Exposure during use is likely to be much higher than exposure due to production and handling. Release from recycling has not been considered.

The figures presented in **Table 2.1** are used to calculate emissions to the environment (including indirect exposure to humans via the environment) from trichloroethylene sold in the EU. Emissions from the use of trichloroethylene as an intermediate are also estimated.

2.3.2 Direct exposures to humans

Occupational exposures have been determined for production, metal cleaning, adhesives, use as an intermediate in HCFC and HFC production and dry cleaning. Other uses as an intermediate or industrial solvent have not been pursued. Consumer exposure has been calculated for spot cleaning of fabrics.

2.4 CONTROLS ON TRICHLOROETHYLENE

Many of the latest degreasing baths used in metal cleaning are fully enclosed, which reduces solvent losses to the workplace and the environment. The extent of use of these modern enclosed vapour degreasing baths varies between member states; it is understood that less than 5% of baths in the UK are of this type, whereas greater than 95% of baths used in Germany are modern enclosed baths. In the UK, there is a trend to the use of fully enclosed degreasing baths mainly driven by the requirements of BATNEEC (Best Available Technique Not Entailing Excessive Cost) under the UK Environmental Protection Act (EPA) 1990.

2.4.1 Legislative controls

Directive 1999/13/EC (the Solvent Emissions Directive) requires EU member states to implement controls on the emissions of volatile organic compounds, which includes trichloroethylene. The major disperse use area for trichloroethylene, metal cleaning, is identified in the Directive. There are emission limit values for the concentration in waste gas (for all emitted compounds together) and limits to the fugitive emissions as a percentage of the solvent input. These vary with the quantity of solvent used on site each year. In each area, new equipment is to meet this standard on installation, while existing equipment has to be brought up to the standard by 2007 - this may increase the use of fully enclosed degreasing baths. The use of

trichloroethylene in adhesives may also be influenced by the requirements of Directive 99/13/EC.

Trichloroethylene is one of the chemicals identified by the Commission of the European Communities as being a List I compound under the Directive on pollution caused by certain dangerous substances discharged into the aqueous environment of the Community (Directive 76/464/EEC). A daughter Directive, 86/280/EEC, has set limit values for the emission of trichloroethylene from industrial plants. An average monthly emission limit value of 10 g/tonne trichloroethylene produced was set for trichloroethylene production plants to be achieved by the end of 1992; the limit value was then reduced to 2.5 g/tonne to be achieved by the end of 1994. The Directive also set a Water Quality Objective of 10 μg/l trichloroethylene in UK surface waters to be achieved by the end of 1992.

Trichloroethylene is classified as a dangerous substance within the meaning of Directive 67/548/EEC and is listed in Annex I of this Directive (see section 1.4).

As an organohalogen compound, trichloroethylene is contained in a List I group of substances under the EC Groundwater Directive (80/68/EEC). Its precise status as an individual substance must be judged on the basis of its persistence, toxicity and bioaccumulation. If confirmed in List I it must be prevented from reaching groundwater.

Trichloroethylene, being a halogenated compound, is a prescribed substance for release to air and to land under the Environmental Protection (Prescribed Processes and Substances) Regulations, 1991.

Information on workplace controls, particularly control limits, has not been obtained for EU countries, since the context in which a particular limit is used and the way it is enforced may be particularly important in determining the level of risk associated with a particular workplace exposure limit. Within the UK, trichloroethylene is subject to a maximum exposure limit (MEL) of 100 ppm, 8-hour time weighted average (TWA) with a short-term exposure limit (STEL), 15-minute reference period, of 150 ppm. The 8-hour TWA is a reference period whereby the occupational exposures in any 24-hour period are treated as equivalent to a single uniform exposure for 8 hours; for the STEL, exposures are averaged over a ten minute period. A skin notation (indicating the potential for absorption through the skin with concerns for subsequent systemic toxicity) has also been applied.

3 ENVIRONMENT

3.1 EXPOSURE ASSESSMENT

3.1.1 Release into the environment

Trichloroethylene has a wide dispersive use and it is likely that the majority of trichloroethylene used will be released to the environment (excluding that which is used as an intermediate). The major release is likely to be to the atmosphere where trichloroethylene has a lifetime of about one week and so is not likely to enter the stratosphere (see Section 3.1.2.1.1). There will be a smaller release to the aquatic environment. For the purposes of this assessment, a release of 90% to air and 10% to water is assumed where no other information is available.

3.1.1.1 Release during production of trichloroethylene

Release to air

A review of air emission factors by the US EPA (1988) gives an emission factor for release of trichloroethylene during production (general process) of 2.07 kg/tonne trichloroethylene produced. The same report also gives an emission factor for release of trichloroethylene during production of trichloroethylene and tetrachloroethylene by oxychlorination of 0.082 kg/tonne produced.

Site-specific information has been provided by European producers regarding release of trichloroethylene to the air during production. The largest single release is 879 tonnes/year, which corresponds to 2.93 tonnes/day over 300 days. This value includes emissions from processing as an intermediate as well as from production, but these cannot be separated. This release will also be used as the emission to the regional environment, as 2.41 tonnes/day over 365 days. The sum of the emissions from the other sites, 78 tonnes/year, will be used for the continental emissions. The releases are shown in **Table 3.1**.

Table 3.1 Release of trichloroethylene to air from production

	Release (kg/day)	Days of release	Release (tonne/year)
Local	2,930	300	879
Regional	2,410	365	879
Continental	214	365	78

Release to water

There is no information given in IUCLID regarding release of trichloroethylene to water during production. In the absence of any information, the Technical Guidance Document (TGD) can be used to estimate a release to wastewater during production of 0.3%.

BUA (1994) cites information from a company producing trichloroethylene. The company has a plant with a capacity of 10,000 tonnes per annum, the effluent from which contains <1 mg/l

trichloroethylene. In 1990, the water capacity of the plant was 8,621 m^3, which gives an emission of trichloroethylene to wastewater of < 8.6 kg per annum. Based on the capacity, this gives a worst-case release estimate of $8.6 \cdot 10^{-4}$ kg/tonne trichloroethylene produced.

Site-specific information has also been provided by companies which produce trichloroethylene regarding release of trichloroethylene to the aquatic compartment during production. Based on the information from various sources, emission factors to water range from $6.4 \cdot 10^{-4}$ to 0.047 kg/tonne for larger plants (>10,000 tonnes). One higher factor was estimated for a small site at 0.83 kg/tonne. As data are available for all production sites, these will be used to calculate local concentrations for the aquatic compartment. For the regional release, the largest individual release from the production sites will be used, which is 13.7 kg/day (over 365 days). The continental release is the sum of releases from the other sites, which is 10.9 kg/day.

Directive 76/464/EEC, on pollution caused by dangerous substances discharged into the aquatic environment of the community, has set a monthly emission limit to water of 2.5 g/tonne produced. This has not been taken into account specifically in this assessment.

3.1.1.2 Release during use of trichloroethylene

Emissions of trichloroethylene may arise from emissions during use as an intermediate or during the manufacture of other substances, during bulk handling and from end use.

It has previously been estimated that western European emissions to air due to end use (degreasing, adhesives, consumer and "other" uses) of trichloroethylene are 60% of total consumption (CEFIC, 1986). The fate of the remaining trichloroethylene is not clear. It may be incinerated or released into other environmental media but it is also possible that it may be recycled.

Release arising from manufacture of other substances and use as an intermediate

Trichloroethylene can be formed during production of tetrachloroethylene by oxychlorination of ethylene dichloride and has been used in the production of PVC. Emission factors are available for these releases (US EPA, 1988) but no information is available about the amounts of these chemicals produced by direct chlorination in western Europe.

Trichloroethylene is used in the production of HFC 134a (1,1,1,2-tetrafluoroethane) which is used as a refrigerant and HCFC 133a (1-chloro-2,2,2-trifluoroethane) which is used to manufacture the anaesthetic halothane. An estimated 45,000 tonnes of trichloroethylene is used as an intermediate in the production of other substances.

Information on emissions to air and water has been provided by a number of companies using trichloroethylene as an intermediate. This information is considered to be sufficiently representative for water to be used in the risk assessment, and a worst-case factor of 0.01% release to water is applied. For air, the emission information is not considered to be sufficiently representative and so the default emission factor of 0.1% from the TGD is used. For comparison, the emission factors estimated from site specific data range from zero to 74 g/tonne.

For local releases it is assumed that 20,000 tonnes of trichloroethylene are used at one site, over 300 days per year. The same site is used to estimate regional emissions; the continental emissions are calculated from the balance of trichloroethylene used as an intermediate, or 25,000 tonnes. Releases to the region and the continent are taken to occur over 365 days per year. The resulting releases are in **Table 3.2**.

Table 3.2 Release of trichloroethylene arising from use as an intermediate

Environment	Emission factor	Volume used as intermediate (t/a)	Release (kg/day)
Local	Air 0.1% Water 0.01%	20,000	67 6.7
Regional	Air 0.1% Water 0.01%	20,000	55 5.5
Continental	Air 0.1% Water 0.01%	25,000	68 6.8

The TGD suggests that releases from use as an intermediate should be added to those from production unless it is clear that the two operations do not take place on the same site. Later in this risk assessment concentrations in water are calculated for specific sites. These cover all sites where production and intermediate use occur together. The local release calculated above for intermediate use will therefore be considered independently.

Release during handling of trichloroethylene

For this assessment, emissive losses from handling are based on site-specific information for a site not producing trichloroethylene. They are considered to apply to sites where trichloroethylene is formulated for solvent use. The worst-case emission factors are 0.4% to air and < 0.00025% to water; these factors are used in the following calculations, with release on 300 days per year to the local environment, and 365 days to the regional and continental environments. For local release a site handling 30,000 tonnes is assumed (this is ~40% of EU non-intermediate use). The same site is used for the regional emissions, and the continental release covers the remaining tonnage (i.e. 77,000 - 30,000 = 47,000 tonnes). The resulting releases are presented in **Table 3.3**.

Table 3.3 Release of trichloroethylene during handling

Environment	Emission factor	Volume handled (t/a)	Release (kg/day)
Local	Air 0.4% Water 0.00025%	30,000	Air 400 Water 0.25
Regional	Air 0.4% Water 0.00025%	30,000	Air 329 Water 0.21
Continental	Air 0.4% Water 0.00025%	47,000	Air 627 Water 0.32

Release during metal degreasing

For the purpose of this assessment, it has been assumed that 82% of trichloroethylene sold is used for metal degreasing and that 70% of trichloroethylene used during metal degreasing is released during use (IUCLID).

It is difficult to describe a "typical" metal degreasing site in terms of its size and the quantity of trichloroethylene used. It is thought that there is a range of plant sizes, and that larger sites (with a greater consumption of trichloroethylene) are more likely to have control measures in place to reduce emissions; smaller sites are less likely to have such control measures. For this assessment, it has been estimated that 20 tonnes of trichloroethylene per year are used at a typical degreasing

plant. This estimate is based on expert judgement from the UK metal finishing industry, industrial waste surveys and information from other countries. The release of trichloroethylene is calculated based on release of 70% of the amount used with 90% of trichloroethylene released going to air and the remaining 10% going to water. The releases to the local environment occur over 300 days per year. Regional and continental releases have been calculated based on release for 365 days per year. The figures are presented in **Table 3.4**.

Table 3.4 Release of trichloroethylene from metal degreasing

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local	70% of trichloroethylene used	20	Air 42 Water 4.6
Regional	70% of trichloroethylene used	6,314	Air 10,898 Water 1,211
Continental	70% of trichloroethylene used	56,826	Air 98,083 Water 10,898

A risk assessment for trichloroethylene has been carried out by ESCA. In this assessment it is stated that 87,274 tonnes per annum represents the amount applied in degreasing. Using these alternative figures and the emission factors used above gives a regional release to air and water of 15,063 kg/day and 1,674 kg/day respectively. The continental release to air and water are 150,637 kg/day and 16,737 kg/day respectively.

For this assessment the figures in **Table 3.4** will be used.

Release during formulation of adhesives

For the purpose of this assessment it is assumed that 9% of trichloroethylene sold (6,930 tonnes/annum) is used for adhesives and associated cleaners. In the UK, 1,000 tonnes/annum of trichloroethylene is used in the production of adhesives at ten to fifteen sites. It has been assumed that this situation is typical of the whole of the EU and that 100 tonnes/annum is used at a typical site. Release of trichloroethylene during formulation of adhesives has been calculated using default release factors in the TGD. The emission factors and releases are given in **Table 3.5**.

Table 3.5 Release of trichloroethylene during formulation of adhesives

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local (300 days)	Air 0.025 Water 0.003 Soil 0.005	100	Air 8.33 Water 1.0 Soil 1.67
Regional (365 days)	Air 0.025 Water 0.003 Soil 0.005	1,000	Air 68.5 Water 8.2 Soil 13.7
Continental (365 days)	Air 0.025 Water 0.003 Soil 0.005	5,930	Air 406 Water 49 Soil 81

It is assumed that 100% of trichloroethylene used as an adhesive is released during use (IUCLID) and that this release is to air. There is no information regarding the type of adhesives trichloroethylene is used in or how much is used at a typical site. Therefore, it has been assumed that there are 100 sites in a region using these adhesives and the use is evenly split between these sites. Releases have been calculated assuming that release occurs on 365 days per year for the local, regional and continental environments. These releases are given in **Table 3.6**.

Table 3.6 Release of trichloroethylene from adhesives use

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local	100% of total used	6.9	Air 19
Regional	100% of total used	693	Air 1,899
Continental	100% of total used	6,237	Air 17,088

Consumer use

Consumer use of trichloroethylene accounts for 6% of sales (4,620 tonnes/annum). No information is available regarding the number of sites where trichloroethylene is used to make consumer products or how much is used at each site. It has been assumed that, as for adhesives, there are ten sites in each region. Releases of trichloroethylene during formulation of consumer products have been estimated using the default emission factors from the TGD (Table A2.1, Appendix A). The emission factors and releases are given in **Table 3.7**.

Table 3.7 Releases from formulation of consumer products

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local	Air 0.025 Water 0.003 Soil 0.005	46.2	Air 3.85 Water 0.46 Soil 0.77
Regional	Air 0.025 Water 0.003 Soil 0.005	462	Air 32 Water 3.8 Soil 6.3
Continental	Air 0.025 Water 0.003 Soil 0.005	4,158	Air 285 Water 34.2 Soil 57

Consumer uses include spot cleaning fabrics, home use, etc. It is thought that consumer use is largely restricted to France, Belgium and Italy, where the product can be purchased, with controls, in some retail outlets. In some retail outlets, sales are controlled by keeping the product in a locked cupboard so consumers must request it.

For this assessment, it is assumed that 100% of the trichloroethylene used in consumer products is released during use (90% to air and 10% to water). This is a diffuse release. The releases of trichloroethylene from use in consumer products are based on a population of 10,000 people in the local environment and 20 million people in the regional environment and are given in **Table 3.8**.

Table 3.8 Release of trichloroethylene from use in consumer products

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local	90% to air 10% to water	0.23	Air 0.58 Water 0.064
Regional	90% to air 10% to water	462	Air 1,139 Water 127
Continental	90% to air 10% to water	4,158	Air 10,253 Water 1,139

Other uses

The remaining 3% of sales (2,310 tonnes/annum) of trichloroethylene are for a range of uses (see Section 2.2.4.) including leather degreasing, as a carrier and general solvent and as an extractant. No information is available about the specific amounts used in each of these areas. In the absence of further information it is assumed in this assessment that 100% of the trichloroethylene used under this heading is released (90% to air, 10% to water). The releases are based on a local population of 10,000 people and a regional population of 20 million people and are given in **Table 3.9**.

Table 3.9 Release of trichloroethylene from other uses

Environment	Emission factor	Volume used (t/a)	Release (kg/day)
Local	90% to air 10% to water	0.12	Air 0.29 Water 0.032
Regional	90% to air 10% to water	231	Air 570 Water 63
Continental	90% to air 10% to water	2,079	Air 5,126 Water 570

Trichloroethylene has been used in industrial dry cleaning, but as noted in Section 2.2.4 there is thought to be little or no use for this purpose in the EU. Dry cleaning machines for use with trichloroethylene are no longer available. Some background information on possible release of trichloroethylene to the environment from dry cleaning has been provided by Finland in the form of measured levels of trichloroethylene in contact water (the waste water just after the machine before dilution to the wastewater system). The trichloroethylene concentrations were between 0.0005 and 2.8 mg/l except for one value of 40 mg/l. The concentration of 40 mg/l in the contact water is from a laundry which produces 1,500 l/annum of contact water. This gives a worst-case release of 0.2 g/day of trichloroethylene to water. The concentration of trichloroethylene in wastewater from the dry cleaning establishment is 6 mg/l and this goes to a wastewater treatment plant with a capacity of 250,000 - 300,000 m^3/day. These results suggest that the release of trichloroethylene to water from dry cleaning would not be environmentally significant.

Trichloroethylene may be released to the environment during disposal. However, as described above, most of the trichloroethylene used is released during use, mainly to the air. Any remaining trichloroethylene which is disposed of would be chlorinated waste and so should be disposed of by proper means.

Chlorinated solvents may be recycled, but only a few companies in the EU are believed to recycle trichloroethylene. Emissions from such sites are expected to be low as recycling takes place in closed systems (see Section 4.1.1.2.2). As the volume of recycled material is small, this will not influence the emission estimates significantly.

3.1.1.3 Natural sources

Modelling studies on trichloroethylene in the atmosphere have indicated that there may be a natural source. McCulloch and Midgley (1996) based their estimate of the anthropogenic release on audited production data from manufacturers worldwide. They used an atmospheric model to match these emissions to observed concentrations, and found that the calculated levels of trichloroethylene were much lower than those measured in remote locations. They suggested two possible explanations, that the rate constant for reaction with OH radicals was too large or that there was a greater release of trichloroethylene to the atmosphere. They concluded that the reaction rate was unlikely be in serious error, and hence that a large (probably natural) further release was required. This would need to be up to one order of magnitude greater than the estimated anthropogenic emissions.

Aucott (1997) carried out modelling studies which predicted a natural source of trichloroethylene contributing 200,000 tonnes per annum to atmospheric emissions in addition to a similar release from anthropogenic sources.

There are reports which suggest that seawater algae may be a natural source of trichloroethylene. Abrahamsson et al. (1995) have investigated the production of trichloroethylene from various macroalgae and a marine microalga. The highest rate of production was for a subtropical macroalgae which produced trichloroethylene at a rate of 3,400 $ng \cdot g \cdot FW^{-1} \cdot h^{-1}$ (where FW is fresh weight). They found that there was a significant difference in the ability to form trichloroethylene between different species. They considered that the activity of algae could be of such a magnitude that it could not be neglected in the global atmospheric chlorine budget.

Appendix B contains calculations on a possible breakdown product of trichloroethylene (dichloroacetic acid), which also indicate the need for a further source to explain the concentrations measured at remote locations.

In view of the uncertainty regarding the source and extent of natural emissions, no attempt has been made to include them in the release estimates in this assessment. The levels of trichloroethylene and dichloroacetic acid in remote regions do indicate the existence of a large source of trichloroethylene other than the known anthropogenic emissions. On the basis of current knowledge this source is most likely to be found in the marine environment. Thus it is unlikely to influence significantly the levels of trichloroethylene found in industrial and rural areas, and hence neglecting the natural contribution on the EU scale is unlikely to have a significant influence on the risk assessment.

3.1.1.4 Summary of releases

Table 3.10 summarises the release of trichloroethylene to the local, regional and continental environments.

Table 3.10 Environmental releases of trichloroethylene

Process	Compartment	Local Release (kg/day)	Regional (kg/day)	Continental (kg/day)
Production	Air Water	2,930 Site-specific basis	2,410 13.7	214 10.9
Manufacture of other substances	Air Water	67 6.7	55 5.5	68 6.8
Handling (formulation as a solvent)	Air Water	400 0.25	329 0.21	627 0.32
Metal degreasing	Air Water	42 4.6	10,898 1,211	98,083 10,898
Formulation of adhesives	Air Water Soil	8.3 1.0 1.67	68.5 8.2 13.7	406 49 81
Use of adhesives	Air	19	1,899	17,088
Formulation of consumer products	Air Water Soil	3.85 0.46 0.77	32 3.8 6.3	285 34 57
Consumer products: use	Air Water	0.58 0.064	1,139 127	10,253 1,139
Others	Air Water	0.29 0.032	570 63	5,126 570
Total	Air Water Soil		17,400 1,432 20	132,150 12,708 138

3.1.2 Environmental fate

3.1.2.1 Degradation

3.1.2.1.1 Abiotic degradation

Photodegradation

A report by the US EPA (1979) stated that direct photo-dissociation of C-C or C-Cl bonds did not appear to contribute to the fate of trichloroethylene in the atmospheric environment. Freitag et al. (1985) irradiated trichloroethylene adsorbed on silica gel with light (>290 nm) and found that 36.8% of the applied trichloroethylene was degraded in 17 hours.

In the atmosphere, trichloroethylene undergoes reactions with hydroxyl radicals, ozone and nitrogen oxide. The dominant degradation process for trichloroethylene in the atmosphere is reaction with hydroxyl radicals which has an estimated half-life of approximately 7 days (US EPA, 1988). The reaction of trichloroethylene with hydroxyl radicals has been investigated by several authors and the results of these studies are summarised in **Table 3.11**.

The rate constant for reaction of trichloroethylene with ozone has been calculated by Atkinson et al. (1982) to be $<3 \cdot 10^{-20}$ $cm^3/mol \cdot sec$. Assuming a concentration of ozone of $1 \cdot 10^{12}$ molecules/cm^3, the lifetime of trichloroethylene is > 390 days.

Table 3.11 Reaction of trichloroethylene with hydroxyl radicals

Reference	Results	Half-life (days)*
Klöppfer et al. (1988)	[OH] = 500,000 mol/cm^3 Rate constant = (2.8-3.0) · 10^{-12} cm^3/mol.sec $t_{½}$ = 5.5 days	5.5
Atkinson (1985)	[OH] = 500,000 mol/cm^3 Rate constant (calc) = 2.36 · 10^{-12} cm^3/mol.sec $t_{½}$ = 6.78 days	6.8
Singh et al. (1981)	Rate constant = 2.2 · 10^{-12} cm^3/mol.sec (300K) residence time = 5.3 days 17.2% loss in 1 day	7.3
Singh et al. (1979)	[OH] = 4 · 10^5 mol/cm^3 Rate constant = 2.3 · 10^{-12} cm^3/mol.sec (265K) residence time = 0.04 years	7.0
Chang and Kaufmann (1977)	Rate constant = 2.37 · 10^{-12} cm^3/mol.sec Calculated lifetime ~ 1 week	6.8
Class and Ballschmiter (1986)	$t_{½}$ = 3-7 days	
Yung et al. (1975)	Rate constant = 3 · 10^{-12} cm^3/mol.sec (296K) t½ ~ 3 days	5.3
Howard (1976)	Rate constant = 2 · 10^{-12} cm^3/mol.sec (296K)	8.0
Güsten et al. (1984)	Rate constant = 2.6 · 10^{-12} cm^3/mol.sec (300K) $t_{½}$ ~ 6 days	6.2

* half-life ($t_{½}$) calculated using [OH] of 5 · 10^5 mol/cm^3

Dilling et al. (1976) studied the reaction between trichloroethylene and nitrogen oxide in a chamber with a UV source of intensity 2.6 times that of natural sunlight at noon in summer in Texas and a humidity of 35-40%. The rates of disappearance of organic compounds were determined by GC/FID. Using initial concentrations of 10 ppm trichloroethylene and 5 ppm NO the time for 50% of the trichloroethylene to disappear ranged from 3.5 to 9.5 hours depending on the light intensity. When initial concentrations of trichloroethylene and NO were increased the time for the disappearance of trichloroethylene decreased. The data suggested that trichloroethylene caused the formation of some highly reactive species other than NO_2 or O_3 in the photochemical reactions. These species appeared to be highly reactive towards trichloroethylene. It should be noted that normal polluted air contains 5-50 ppb NO, which if the reaction is second order would mean half lives of around 1,000-10,000 hours.

Under smog conditions, 66% of trichloroethylene was degraded after 140 minutes (Gay et al., 1976). The study was carried out in two chambers. In one chamber *in situ* IR spectroscopic

methods were used for measurement; in the other wet chemical and GC methods were used. The initial concentration of trichloroethylene was 3.45 ppm, the ratio of trichloroethylene to NO_2 being 2.66. After 140 minutes irradiation, 66% of trichloroethylene had reacted. Spectral data showed the presence of phosgene and dichloroacetyl chloride.

Trichloroethylene (1 mg/ml) in water was degraded by 52-56% after one year in the dark and by 70-79% in the presence of sunlight (Dilling et al., 1975).

Photodegradation products

Some authors have suggested that dichloroacetyl chloride (DCAC) is the major atmospheric degradation product of trichloroethylene. Chloroacetyl chlorides can react further to form chloroacetic acids which are toxic to many plants and some have been used as herbicides (see Appendix B).

Snelson et al. (1978) estimated the half-life for removal of trichloroethylene by photooxidation at the double bond to be 0.001 year (8.8 hours). The major reaction product was DCAC (70%) with smaller amounts of phosgene (12%), formyl chloride (8%) and hydrogen chloride (10%) also being formed. Kinetic data could not be obtained for DCAC due to experimental difficulties but assuming the lifetime is similar to that for mono- and trichloroacetyl chlorides the half-life in the troposphere was estimated to be more than 20 years and in the stratosphere more than 109 years. (Note that these half-lives refer to the photo-stability of the species; hydrolysis of the chloroacetyl chlorides is much more rapid than this (see Appendix B)). Snelson et al. also investigated the heterogeneous "rain-out" of the acetyl chlorides. Preliminary data for trichloroacetyl chloride (TCAC) indicated that "rain-out" would probably be an effective removal mechanism for this species from the atmosphere.

The gas-phase chlorine-photosensitised oxidation of trichloroethylene was studied by Huybrechts and Meyers (1967). DCAC was found to be the main reaction product and the reaction mechanisms were established.

Gay et al. (1976) investigated the photooxidation of trichloroethylene in air in the presence of nitrogen dioxide with UV light. DCAC was found to be the major reaction product together with formyl chloride and phosgene.

Jacoby et al. (1994) carried out a series of experiments to study the products, intermediates, mass balances and reaction pathways for the oxidation of trichloroethylene in air via heterogeneous photocatalysis. It was found that the reaction pathway through the DCAC intermediate was significant during photocatalytic oxidation of trichloroethylene. DCAC, phosgene, carbon dioxide, carbon monoxide and hydrogen chloride were observed in the effluent of photocatalytic reactors with thin films of titanium dioxide catalyst.

Recently, however, other authors have suggested that the degradation pathway which produces DCAC requires that trichloroethylene reacts with chlorine atoms produced by the reaction of trichloroethylene with hydroxyl radicals. For example, Tuazon et al. (1988) investigated the kinetics and products of the homogenous gas-phase reactions of the hydroxyl radical with trichloroethylene using ethane as a scavenger for the chlorine atoms produced in these hydroxyl radical reactions. The major products observed were formyl chloride (HC(O)Cl) and phosgene ($COCl_2$) with some DCAC. Kinetic and product data obtained showed that chlorine atoms are generated from the reaction of hydroxyl radicals with trichloroethylene. In the absence of a chlorine atom scavenger, significant yields of DCAC were found. The authors suggested that the decrease in yields of chloroacetyl chlorides in the presence of chlorine atom scavengers shows that these compounds are products of chlorine atom reaction with the chloroethylenes.

Itoh et al. (1994) have studied the reaction of trichloroethylene with hydroxyl radicals and found that DCAC is a major reaction product. However, the authors suggested that in the real atmosphere chlorine atoms from the reaction of trichloroethylene and hydroxyl radicals would not be available to react with trichloroethylene since there are many chemical species, such as hydrocarbons, capable of scavenging chlorine atoms.

Franklin (1994) has also suggested that in the real atmosphere concentrations of chlorinated ethylenes are much less than in laboratory studies, so chlorine atoms produced are less likely to react with the chlorinated ethylene than with other species present.

Sidebottom and Franklin (1996) have recently reviewed the atmospheric fate and impact of chlorinated solvents and argued that it is unlikely that chloroacetic acids are the major products of degradation. This is based on a review of various studies of degradation of chlorinated solvents, particularly tetrachloroethylene but including trichloroethylene. Sidebottom and Franklin (1996) have suggested that the formation of TCAC and DCAC from atmospheric degradation of tetrachloroethylene and trichloroethylene respectively depends on the relative atmospheric importance of chlorine atom addition versus hydroxyl radical addition. It will also depend on the products of the hydroxyl-radical addition pathway. Sidebottom and Franklin (1996) reported that recent studies have confirmed that, in systems free of chlorine atoms, the yield of C2 products formed in oxidation of trichloroethylene is negligible.

Appendix B contains estimates of the amount of dichloroacetic acid (DCA) which could be formed from the breakdown of trichloroethylene based on the relative reaction rates with hydroxyl and chlorine radicals and the subsequent reaction steps. The yield of dichloroacetic acid is estimated as 0.9%; it is recognised that this estimate is subject to some uncertainty. The Appendix also estimates the levels of DCA in the environment which could result from this. Background levels have been measured in remote locations. Estimates of these concentrations based on a yield of 0.9% and the estimated anthropogenic emissions of trichloroethylene are much lower than the measured values. Using the estimates of total trichloroethylene flux from McCulloch and Midgely (1996) (which are around eight times higher than anthropogenic emissions for the northern hemisphere and over one hundred times higher for the southern hemisphere) gives DCA concentrations much closer to those measured.

The conclusion is that trichloroethylene photodegradation can lead to the formation of dichloroacetic acid in the air, and that the concentrations of dichloroacetic acid measured in precipitation are likely to arise from this source.

Hydrolysis

US EPA (1979) have reported that trichloroethylene is not hydrolysed under normal conditions and Jeffers et al. (1989) have calculated a half-life for abiotic degradation of trichloroethylene of 1.3 million years at pH 7 and 25°C. Dilling et al. (1975) have measured a half-life for abiotic degradation (oxidation and hydrolysis) of trichloroethylene in a closed system in the dark of 10.7 months at 25°C. This process is, therefore, not likely to be an important removal process for trichloroethylene. The half-life for hydrolysis of trichloroethylene has been measured at 25°C and various pHs (Korte and Greim, 1981). At pH 3 the half-life was 2,809 hours (117 days), at pH 7 no hydrolysis was observed and at pH 9 the half-life was 3,489 hours (145 days).

Summary

Trichloroethylene undergoes reactions with hydroxyl radicals in the atmosphere. The half-life of trichloroethylene due to this reaction is about one week. Trichloroethylene also reacts with ozone

and nitrogen oxide in the atmosphere but with longer half lives under environmental conditions. Trichloroethylene can also react with chlorine atoms in the atmosphere. Overall, trichloroethylene is rapidly degraded in the atmosphere. Hydrolysis is not likely to be a significant removal process for trichloroethylene.

The major reaction products in the absence of chlorine atoms are formyl chloride and phosgene. However, the major product of the reaction with chlorine atoms is dichloroacetyl chloride (DCAC) which is rapidly hydrolysed to form dichloroacetic acid (DCA). Based on the available information it is concluded that the DCA found in precipitation is formed through the breakdown of trichloroethylene in the atmosphere.

The levels of trichloroethylene and dichloroacetic acid in remote regions indicate the existence of a large source of trichloroethylene other than the known anthropogenic emissions. On the basis of current knowledge this source is most likely to be found in the marine environment, and is unlikely to influence significantly the levels of trichloroethylene and dichloroacetic acid found in industrial and rural areas.

3.1.2.1.2 Biodegradation

Aerobic degradation

The results of various studies to investigate the biodegradation of trichloroethylene in activated sludge are given in **Table 3.12**.

Table 3.12 Aerobic degradation of trichloroethylene in activated sludge

Test conditions	Test results	Reference
Closed bottle test (OECD guideline 301D). 2-10 mg/l trichloroethylene incubated with industrial activated sludge	19% degradation after 28 days	Rott et al. (1982)
Modified MITI test (OECD 301C). 100 mg/l trichloroethylene incubated with activated sludge	2.4% degradation after 14 days	CSCL (1992)
0.05 mg/l trichloroethylene applied to activated sludge	3.4% biodegraded in 5 days	Freitag et al. (1985)

Nielsen et al. (1992) investigated the degradation potential for a mixture of eight organic trace contaminants using *in situ* microcosms. The study was carried out in a shallow sand and gravel aquifer which was partly influenced by a leachate plume from a landfill. Four microcosms were in the aerobic zone of the pollution plume and two in the unpolluted zone. The microcosms were loaded with organics and analysed at intervals over 90 days. Chlorinated aliphatics including trichloroethylene were not degraded under aerobic conditions.

Tabak et al. (1981) studied the degradability and rate of acclimation of trichloroethylene in a static-culture flask screening procedure. A 7-day static incubation with 5 and 10 mg/l trichloroethylene at 25°C in the dark, followed by three weekly subcultures, was carried out using settled domestic wastewater as microbial inoculum. Glass-stoppered vials were used during incubation to minimise possible losses through volatilisation and volatility controls were analysed to determine loss of substrate through this route. The loss of trichloroethylene from the incubation with 5 mg/l was 64% in the original culture and 73%, 82% and 87% in the three successive subcultures. The loss due to volatilisation (after 10 days incubation at 25°C) was 29%. Following incubation with 10 mg/l trichloroethylene, the loss was 38% in the original

culture and 56%, 76% and 84% in the subcultures; loss through volatilisation was 22%. The overall summary was that significant degradation occurred with gradual adaptation.

Table 3.13 gives the results of experiments to investigate the degradation of trichloroethylene in the presence of single culture bacteria.

Table 3.13 Aerobic degradation of trichloroethylene by single culture bacteria

Test conditions	Test results	Reference
Trichloroethylene inoculated with *Methylosinus trichosporium* in presence of oxygen and formate	Trichloroethylene 100% degraded in 20 hours	Oldenhuis et al. (1980)
Mycobacterium vaccae incubated with 3.3 mg/l trichloroethylene in presence of propane	Trichloroethylene 10% degraded after 2 hours. After 24 hours incubation, *M.vaccae* cleared nearly all (99%) of trichloroethylene from reaction vials.	Wackett et al. (1989)
Nitrosomonas europae incubated with 1 mg/l trichloroethylene for 24 hours	Trichloroethylene 48% degraded in absence of ammonia Trichloroethylene 94% degraded in presence of ammonia	Vanelli et al. (1990)

Vanderberg et al. (1995) investigated the biodegradation of trichloroethylene by *Mycobacterium vaccae* which had been grown with propane as the sole carbon and energy source. *M. vaccae* was found to be able to mineralise limited amounts of trichloroethylene. During a 48-hour incubation with trichloroethylene (0.7 mM), the addition of toluene (500 mM) effected a 50% increase in mineralisation of ^{14}C trichloroethylene while the addition of propane (50% of the total atmosphere in the vessel) had a negative effect on trichloroethylene oxidation.

Wilson and Wilson (1985) fed trichloroethylene to mixed cultures in a soil column exposed to natural gas 0.6% v/v (77% of natural gas was methane). Three weeks were allowed for acclimation before the soil was fed water containing 150 μg/l trichloroethylene. After two weeks feeding with trichloroethylene, less than 5% of the applied trichloroethylene passed through the soil. Breakthrough of trichloroethylene increased when the column was poisoned with sodium azide which confirmed that the low level of breakthrough was due to biological activity. In an earlier study without exposure to methane, no significant degradation of trichloroethylene was observed. Dosing of a second soil column with ^{14}C-labelled trichloroethylene showed that trichloroethylene is degraded to carbon dioxide.

Henson et al. (1989) incubated trichloroethylene (200-300 μg/l) in the presence of methane with a mixed soil culture. After 8 days, 10% of trichloroethylene remained; after 30 days, 100% of the trichloroethylene was degraded.

Sub-surface sediments were incubated with trichloroethylene (50 mg/l), and with either methanol or propane for 10-30 days (Phelps et al., 1988). After 10 days incubation with methanol, 25% trichloroethylene was degraded and after 30 days 95% was degraded. After 10 days incubation with propane, 0% was degraded, and after 30 days 45% was degraded.

Bacteria indigenous to a trichloroethylene-contaminated site were exposed to 0.56 and 6.7 mg/l trichloroethylene (McClellen et al., 1989). Lag times of 14 and 18 days were observed for the low and high concentration microcosms respectively. The average trichloroethylene concentration for all samples taken after 18 days (for the high concentration) represented a 51% removal of trichloroethylene. For the low concentration samples, the average trichloroethylene

concentration for samples taken after 14 days represented a 61% removal of trichloroethylene. However, replicate samples showed significant variation in the degree of removal.

Hopkins et al. (1993) carried out a study of *in situ* co-metabolic degradation of trichloroethylene by injection of phenol and oxygen in a confined aquifer. With initial concentrations of trichloroethylene between 62 and 500 μg/l and phenol and oxygen concentrations of 12.5 and 35 mg/l respectively, first order removal of trichloroethylene was 88%. When 1,000 μg/l trichloroethylene was used, removal was lower (77%) but increased when phenol was increased to 25 mg/l. Laboratory studies showed increased degradation of trichloroethylene in the presence of formate, lactate and to a small extent acetate.

Mu and Skow (1994) incubated a silt loam soil with trichloroethylene and toluene in the dark at 28°C. Indigenous microbial populations in soil degraded trichloroethylene in the presence, but not the absence, of toluene after a 60-80 hour lag period. As the initial toluene concentration was increased the numbers of trichloroethylene and toluene degraders and the percentage removal of trichloroethylene were both observed to increase. The lag period also increased with increasing toluene concentration. As the initial trichloroethylene concentration increased from 1 to 20 μg/ml, the numbers of toluene and trichloroethylene degraders and the rate of toluene degradation decreased and no trichloroethylene degradation occurred. No toluene or trichloroethylene degradation occurred at an initial trichloroethylene concentration of 50 μg/ml.

Fuller et al. (1995) measured the potential for microbial communities in contaminated and uncontaminated soil materials to aerobically degrade trichloroethylene and toluene. The biodegradation of trichloroethylene (1 μg/ml) and toluene (20 μg/ml) was measured in samples of material from contaminated and uncontaminated sites. Substantial trichloroethylene degradation only occurred in the uncontaminated sample after 220 hours and trichloroethylene did not degrade at all in the absence of toluene. The results indicated that mineral nutrients limited the rate of trichloroethylene and toluene degradation by indigenous populations.

Labelled trichloroethylene (80 ppb) was incubated with a methane-utilizing mixed marsh culture in adapted sludge (Fogel et al., 1986). The trichloroethylene was removed after 2 days with 57% biotransformed as CO_2 (23%) and biomass (34%). Incubation of the same culture with 0.65 mg/l trichloroethylene was also carried out and 69% degradation occurred after 96 hours.

Barrio-Lage et al. (1987) studied the depletion (i.e. the removal mechanism was not determined) of trichloroethylene in microcosms containing water and three types of natural sediment (muck, sand and rock). The organic carbon contents for the muck, sand and rock were 25%, 2% and < 1% respectively, and the sand had already been exposed to trichloroethylene through a major spill from a storage tank. The muck and sand had similar depletion rates and the depletion in the rock microcosm was slower. The results suggest that depletion depends on the organic content of the sediment and the microbial biomass.

Hopkins and McCarty (1995) investigated the *in situ* biotransformation of chlorinated hydrocarbons in groundwater with phenol and toluene as the primary substrates. The test zone represented a groundwater travel time of less than 2 days and removal efficiencies for 250 μg/l trichloroethylene were > 90% when either 9 mg/l toluene or 12.5 mg/l phenol was used.

No degradation of trichloroethylene in seawater was observed under aerobic conditions (Jensen and Rosenberg, 1975).

A series of volatile organic compounds (VOCs) was added to a water column (Narragansett Bay seawater) of a mesocosm at concentrations typical of a moderately polluted estuary (0.2-4 μg/l) (Wakeham et al., 1983). The concentrations were followed for up to two months under

experimental conditions simulating winter, spring and summer. Similar behaviour was observed in tanks with and without $HgCl_2$ with half-lives of 10.7 and 8.6 days respectively. This suggests that trichloroethylene is resistant to microbial degradation in seawater.

Anaerobic degradation

Results of some investigations of degradation of trichloroethylene under anaerobic conditions are given in **Table 3.14**.

Methanogens were incubated with 8 μg/l trichloroethylene under static test conditions at 35°C (Baek and Jaffe, 1989). Trichloroethylene was 18% degraded after 28 days in the presence of a methanogenic culture. In the presence of a culture containing non-methanogenic fermenters and methanogens, trichloroethylene was 63% degraded after 28 days.

Table 3.14 Anaerobic degradation of trichloroethylene

Test conditions	Test results	Reference
Trichloroethylene (50 mg/l) incubated in subsurface sediment (10g) with methanol	25% degradation after 10 days, 95% degradation after 30 days	Phelps et al. (1988)
Trichloroethylene (50 mg/l) incubated in subsurface sediment (10g) with glucose	65% degradation after 10 days, 99% degradation after 30 days	Phelps et al. (1988)
Trichloroethylene (200 μg/l) incubated with batch bacterial cultures under methanogenic conditions	Trichloroethylene removed slowly with a reduction of 40% after 8 weeks	Bouwer and McCarty (1983)
Trichloroethylene incubated in methanogenic column under anaerobic conditions	Influent concentration of 300 μg/l trichloroethylene reduced to < 5 μg/l at 10 cm part of column after 10 days	Vogel and McCarty (1985)

Incubation of trichloroethylene with a contaminated soil was carried out at 20°C (Pavlostathis and Zhuang, 1993). Reductive dechlorination of trichloroethylene was observed under sulphate-reducing and fermentative/methanogenic conditions. Low levels of vinyl chloride were produced.

The rate of removal of trichloroethylene by methanogens was studied using a pH-stat system (Rhee and Speece, 1992). In a formate enrichment culture using a sludge retention time of 20 days, 96% of trichloroethylene was degraded. In acetate and propionate cultures, using a sludge retention time of ten days, the removal rates were 98% and 99%. Biodegradation was the predominant removal mechanism for trichloroethylene in all cultures, and 1,1-dichloroethylene was detected as an intermediate product. Vinyl chloride was not detected in either liquid or gaseous effluent.

Microcosms of an authentic aquifer known to support methanogenesis were incubated with trichloroethylene (Wilson et al., 1986). The aquifer material was obtained from a site adjacent to a landfill. After 40 weeks, removal of trichloroethylene in one microcosm was similar to the control but in the others trichloroethylene was reduced to 21%, 9% and <2% of controls. A long lag period of 16 weeks was required before disappearance of trichloroethylene relative to controls was observed.

Trichloroethylene is biotransformed by freshwater sediment microbiota in sealed static microcosms (Parsons and Lage, 1985).

Breakdown products - formation of vinyl chloride from trichloroethylene

Trichloroethylene is a contaminant of many groundwater sites. There are concerns that vinyl chloride is formed in groundwater as a result of anaerobic degradation of trichloroethylene. Background concentrations of vinyl chloride are given in the IUCLID database for vinyl chloride. Vinyl chloride concentrations of <1-110 μg/l have been found in groundwater of 51 wells in Berlin and a survey of groundwater supplies in the USA showed that vinyl chloride was only detected (detection limit 1 μg/l) in 0.74% of supplies. At contaminated sites, it was found at much greater levels (between < 0.3 and 1,040 μg/l). Vinyl chloride is thought to be formed from tetrachloroethylene and trichloroethylene by soil bacteria under reducing conditions. Up to 2,800 μg/l vinyl chloride was found in contaminated groundwater together with tetrachloroethylene in a narrow stretch of groundwater. This contamination was traced back to a dry cleaning shop.

Other studies are also available which indicate contamination of groundwater supplies with vinyl chloride where trichloroethylene is also a contaminant. For example, in the USA, health assessments have been carried out for various sites involving a determination of groundwater pollutants. Near the Rose Disposal Pit, used for dumping of waste oils and solvents, there was a groundwater plume containing vinyl chloride (ATSDR, 1989a). Off-site levels of trichloroethylene and vinyl chloride were 100 μg/l and 17 μg/l respectively. At a site previously used for metal casting, where trichloroethylene had been used as a degreaser, trichloroethylene concentrations on-site up to 1,300 ppb were found for 1981-1986, and up to 138 ppb for 1987-1988. At the same site, vinyl chloride concentrations for 1981-1986 and 1987-1988 were up to 235 ppb and up to 213 ppb respectively (ATSDR, 1989b). Near a site contaminated with waste oil and other fluids, trichloroethylene levels up to 35 ppb and vinyl chloride levels up to 37 ppb were found (New Jersey State Dept. of Health, 1995).

Various studies have been carried out to demonstrate the degradation of tetrachloroethylene and trichloroethylene in groundwater. McCarty (1996) reported that there is ample evidence to suggest that anaerobic reductive transformation of chlorinated organics occurs frequently. The major environmental requirement is the presence of sufficient concentrations of other organics that can serve as electron donors; this requirement is often fulfilled in aquifers. McCarty showed a potential transformation pathway for tetrachloroethylene via trichloroethylene, dichloroethene and vinyl chloride to ethene and possibly to ethane. Complete reductive transformation of tetrachloroethylene and trichloroethylene to ethene was reported to generally require conditions suitable for methane fermentation. This needs enough organic contaminant to reduce all the oxygen, nitrate, nitrite and sulphate present. Reduction can also occur under sulphate-reducing conditions. McCarty has also suggested that natural attenuation of vinyl chloride can occur at the aerobic fringes of plumes with methane and vinyl chloride present or where sufficient iron (III) is present. McCarty has also reported that many reports on the natural biological attenuation of chlorinated aliphatic hydrocarbons have shown conversion of tetrachloroethylene and trichloroethylene to non-chlorinated end-products.

Gossett and Zinder (1996) reported that results from many laboratory and field studies show that chlorinated ethenes can be sequentially reductively dechlorinated under anaerobic conditions ultimately yielding ethene. The process requires an electron donor and the completeness of conversion to ethene is highly variable from site to site. Gossett and Zinder suggested that the success of natural attenuation depends on the type of dechlorinator (cometabolic or direct) present and the relative supply of electron donors compared with the supply of chlorinated ethene present.

Major et al. (1991) have carried out field and laboratory investigations to determine whether vinyl chloride was being mineralised or biotransformed to innocuous end products. The study was carried out at a chemical transfer facility where tetrachloroethylene had been stored. Free and dissolved tetrachloroethylene and dechlorination intermediates of tetrachloroethylene were found in groundwater below the facility. Groundwater and sediment samples were collected and analysed and microcosm studies were carried out. The authors suggested that if dechlorination products of tetrachloroethylene were persisting at the site, greater down-gradient transport of these products would be expected. However, at this site there was a good degree of overlap of tetrachloroethylene, trichloroethylene, cis-1,2-dichloroethene and vinyl chloride indicating that dechlorination of these compounds is occurring. Microcosm studies showed that over time tetrachloroethylene was dechlorinated to trichloroethylene, then to *cis*-dichloroethene, vinyl chloride and finally to ethene.

Freedman and Gossett (1989) also carried out studies of enrichment cultures of tetrachloroethylene and trichloroethylene degrading microorganisms and found that under methanogenic conditions, mixed cultures are able to completely dechlorinate these compounds to ethylene. Radiotracer studies with ^{14}C-tetrachloroethylene indicated that ^{14}C-ethene was the final product with no significant conversion to $^{14}CO_2$ or $^{14}CH_4$. It was found that an electron donor was required to sustain reductive dehalogenation.

Vogel and McCarty (1985) found evidence of tetrachloroethylene transformation by reductive dehalogenation to trichloroethylene, dichloroethene and vinyl chloride under anaerobic conditions. Trichloroethylene and vinyl chloride were found to be the major metabolites.

De Bruin et al. (1992) studied the reductive dechlorination of tetrachloroethylene in a column filled with anaerobic sediment from the Rhine and an anaerobic sludge. They found that tetrachloroethylene was dechlorinated to ethene in the presence of an electron donor which was required to prevent accumulation of chlorinated intermediates. De Bruin et al. suggested that several different microorganisms may be needed for complete dechlorination of tetrachloroethylene.

Maymo-Gatell et al. (1995) found that tetrachloroethylene could be dechlorinated to vinyl chloride and ethene using methanol as an electron donor.

Beeman et al. (1994) carried out a two-year investigation of *in situ* microbial reductive dehalogenation of tetrachloroethylene to ethene and ethane in an aquifer underlying a plant near Victoria, Texas. They first demonstrated that tetrachloroethylene could be dechlorinated. Then tetrachloroethylene and daughter products were degraded to ethene and ethane *in situ* under sulphate reducing conditions with benzoate as an electron donor. Initial concentrations of tetrachloroethylene, trichloroethylene and dichloroethylene in the pilot site were approximately 1,700, 535 and 385 ppb respectively, with vinyl chloride not detected. After two years concentrations of tetrachloroethylene, trichloroethylene, dichloroethylene, vinyl chloride and chloroethane were below detection limits. In some wells, ethene and ethane were detected at near stoichiometric concentrations based on chlorinated hydrocarbons in the feed stream. The authors speculated that microbial succession may be the mechanism which completes reductive dehalogenation of dichloroethene and vinyl chloride to ethene and ethane. Therefore, a range of bacteria rather than a single species is needed to completely dehalogenate tetrachloroethylene.

These various studies show that trichloroethylene can be transformed to vinyl chloride under anaerobic conditions and that one mole of trichloroethylene could produce one mole of vinyl chloride. However, several studies have also shown that the reductive dechlorination would continue to form ethene and ethane. Also, vinyl chloride is also produced from other chlorinated

species, i.e. dichloroethene and tetrachloroethylene. Therefore, it is difficult to quantify the amount of vinyl chloride that is formed as a result of trichloroethylene contamination. Possible risks to the environment from this are not addressed in this risk assessment report.

Summary

The results of biodegradation tests are variable. Trichloroethylene is not readily biodegradable according to the OECD test (301D) and is only slightly degraded in aerobic studies. However, biodegradation seems to occur when trichloroethylene is incubated with bacterial cultures in the presence of another substrate such as propane or formate or with adapted cultures. For the purpose of this assessment trichloroethylene will be considered to be non-degradable (as a worst-case assumption). The degradation rates in wastewater treatment plants (WWTPs) and in environmental media are taken from EUSES with biodegradation set to "not biodegradable" and are as follows:

WWTP	$0\ d^{-1}$
Surface water	$1.39 \cdot 10^{-6}\ d^{-1}$
Sediment	$6.93 \cdot 10^{-8}\ d^{-1}$
Soil	$6.93 \cdot 10^{-7}\ d^{-1}$

Trichloroethylene does seem to be degraded in the presence of anaerobic cultures, although this also seems to occur more readily in the presence of other substrates.

3.1.2.2 Environmental distribution

3.1.2.2.1 Adsorption

Laboratory studies have been carried out to investigate the adsorption of trichloroethylene on various types of soil and the results are given in **Table 3.15**.

Table 3.15 Adsorption of trichloroethylene on various soils

Reference	Soil and conditions	K_{oc}
Rogers and MacFarlane (1981)	Clay loam soil (2.6% organic carbon), Clay loam soil (1.8% organic carbon)	188 (average of 2 soils)
Seip et al. (1986)	Forest soil (0.2% organic carbon), 0.5 μg/g trichloroethylene, pH 5.6	72.5
Seip et al. (1986)	Agricultural soil (2.2% organic carbon), 0.5 μg/g trichloroethylene, pH 7.4	95.8
Seip et al. (1986)	Forest soil (3.7% organic carbon), 0.5 μg/g trichloroethylene, pH 4.2	142
Abdul et al. (1987)		65
Friesel et al. (1984)	Average of 18 values for various soils	58 (K_{SOM})
Grathwohl (1990)	Various soils and sediments	123
Scheubel (1984)	Three soils (organic carbon content 0.76-3.56%)	127-183
Korte and Freitag (1984)	Three soils (organic carbon content 0.76-3.56%) OECD Test Guideline no. 106 (12/5/81)	316-921
Lee et al. (1989)	Agricultural soil (0.3% organic carbon), 0.5 μg/g trichloroethylene, pH 5.4. Duplicate experiments, one treated with HDTMA [a)]	Untreated 0 Treated 30

[a)] HDTMA = hexadecyltrimethylammonium, an organic cation displaying high sorptive uptake of common groundwater contaminants.

Wilson et al. (1981) added trichloroethylene to a sandy soil (0.087% organic carbon) at two concentrations. The temperature was 20°C and the pH was 6.4. When 0.9 mg/l trichloroethylene was added, 28 ± 1% was found in the effluent, 58 ± 14% was volatilised and 14 ± 15% was degraded or not accounted for. When 0.18 mg/l trichloroethylene was used, 21 ± 13% was detected in the effluent, 88 ± 18% was volatilised and 9 ± 11% was unaccounted for.

One field trial has been carried out using a fine sandy loam soil with an organic carbon content of 0.13% (Wilson et al., 1981). The trial was carried out at 20°C and the retardation factor relative to chloride determination was 1.6-3.1. Results of these experiments suggest that adsorption of trichloroethylene onto soil will not occur to a significant extent.

A calcium saturated clay was found to be ineffective as an adsorbent (Rogers and MacFarlane, 1981) with only 17% of available trichloroethylene being adsorbed. Only 6% and 4% of available trichloroethylene was adsorbed by two soils with organic carbon contents of 2.6% and 1.8%.

Grathwohl and Reinhard (1993) investigated the desorption of trichloroethylene in Santa Clara aquifer material. The rate and equilibrium of trichloroethylene sorption were studied using batch experiments and sorption isotherms were consistent with the Freundlich isotherm. Desorption experiments using columns packed with wet or dry aquifer material were carried out over a few days and only 30-50% of the sorbed trichloroethylene was removed.

Parker et al. (1993) studied the behaviour of tap water dosed with trichloroethylene in a pilot scale activated sludge treatment plant. Sorption was found to be insignificant.

Wilmanski and van Breeman (1990) investigated the interactions between trichloroethylene and humic substances during simultaneous adsorption from groundwater on activated carbon. The results indicated that the presence of humic substances in water significantly suppresses trichloroethylene adsorption. No influence of trichloroethylene on adsorption of humic substances was found.

Fares et al. (1995) investigated the rates for desorption of trichloroethylene from powdered soils. Standard reference materials were used and soil samples were saturated with trichloroethylene prior to desorption. Desorption was found to be relatively rapid during the first hour and short-term kinetics (< 24 hours) are apparently dominated by trichloroethylene desorption from surface sites. The desorption rate is strongly dependant on soil properties with linear dependence on inverse particle size.

Alvarez-Cohen et al. (1993) investigated the sorption of trichloroethylene on to a synthetic zeolite. Trichloroethylene concentrations between 0.4 and 150 mg/l were used with 1 or 2 mg of zeolite and equilibrium was reached in less than 1 day.

The adsorption and desorption characteristics of trichloroethylene in a sandy loam soil, an organic top soil and a peat moss were determined (Zytner, 1992). The average soil-water partition coefficient was 118 which indicates that trichloroethylene is highly mobile in soil.

The K_{oc} value calculated from the octanol-water partition coefficient (log K_{ow} = 2.29) using the equation on the TGD (hydrophobics) is 90.2 (log value = 1.96). The available measured values range from close to zero to 921. Rather than try to derive a single value from those available statistically (as some of the values presented are already mean values themselves) or select one of the experimental results, the predicted value has been taken as representative. It has been used to calculate the various water-solid partition coefficients using the methods in the TGD. These are:

$K_{soil\text{-}water}$	= 2.99	Soil-water partition coefficient
$K_{susp\text{-}water}$	= 3.16	Suspended matter-water partition coefficient
$K_{sed\text{-}water}$	= 3.06	Sediment-water partition coefficient

A sediment-water partition coefficient of 2.1 has been calculated by the US EPA (1982).

3.1.2.2.2 Volatilisation

Dilling et al. (1975) found that the half-life for a stirred water body with an initial trichloroethylene concentration of 1 mg/l was between 19 and 24 minutes. Dilling (1977) measured half-lives for volatilisation of trichloroethylene from a stirred water body 6.5 cm deep with an initial trichloroethylene concentration of 1 mg/l. The half-life was found to be between 17.7 and 23.5 minutes.

The half-life in another aqueous solution at 20°C was found to be 18 hours/m depth of solution (Geyer et al., 1985).

The half-life for volatilisation from the river Main was calculated by film theory to be 5.7 days (Brüggeman and Trapp, 1988).

Volatilisation of trichloroethylene from water 1 m deep at 20°C was measured and the half-life for volatilisation was also calculated (Korte and Greim, 1981). The calculated half-life was 6.4 hours and the measured half-life was 18 hours.

The Henry's Law constant calculated from the ratio of vapour pressure to solubility is $1.03 \cdot 10^3$ $Pa \cdot m^3 \cdot mole^{-1}$, and this value has been used in the EUSES calculations.

3.1.2.2.3 Summary

Various laboratory and field studies of adsorption of trichloroethylene on various materials have been carried out. It was found that trichloroethylene is highly mobile in soils and adsorption is not likely to be a significant removal process. The K_{oc} value calculated from the octanol water partition coefficient is 90.2 (log value = 1.96). This value has been used to calculate the various water - solid partition coefficients using the methods in the TGD. Volatilisation was found to occur readily from surface waters with half lives between 3 and 18 hours for water 1 m deep. Therefore, volatilisation is likely to be a significant removal process.

3.1.2.3 Bioaccumulation

Bluegill *Lepomis macrochirus* (a freshwater fish) was exposed to 8.23 µg/l trichloroethylene for 14 days at 16°C in a closed, flow-through system (Barrows et al., 1980). The concentration of trichloroethylene (as ^{14}C-label) was monitored to steady state in water and fish. The bioconcentration factor (BCF) was 17 and the half-life in tissue was less than 1 day.

Korte and Freitag (1984) measured the bioaccumulation of tetrachloroethylene in Zebra fish *Brachydanio rerio* in a semi-static test and found the average bioconcentration factor to be 19.

Freitag et al. (1985) measured the bioconcentration factor in another freshwater fish, Golden Orfe *Leuciscus idus melanotus*, when exposed to 50 µg/l ^{14}C-labelled trichloroethylene for

3 days in a closed, static test. The bioaccumulation factor was 90. Freitag et al. (1985) also measured the bioaccumulation factor in activated sludge to be 990.

Geyer et al. (1984) measured the bioconcentration factor of trichloroethylene to the alga *Chlorella fusca vacuolata* to be 1,160. Smets and Rittmann (1990) have measured bioconcentration factors of trichloroethylene in several species of algae when exposed to 1-1,000 μg/l for five days. The ranges of bioconcentration factors calculated using a Freundlich isotherm were 1.4-5.37 for *Chlorella vulgaris*, 1.51-4.27 for *Scenedesmus quadricauda* and 1.5-3.44 for *Selenastrum capricornutum* with BCF increasing with concentration (using a linear isotherm the BCF values were 2.23, 2.21 and 2.26 respectively). These values are expressed as l/kg.

Bioconcentration factors were measured for the gills, muscle, liver, spleen, kidney and gonad of fish (van de Graaff, 1986). The highest bioconcentration factor measured was 210 for the spleen.

Trichloroethylene did not exhibit any bioaccumulation in a flow-through test with carp (*Cyprinus* species) (Scheubel, 1984) although the exposure concentrations dropped below 80% of the nominal concentration.

In summary, bioaccumulation does not appear to occur to a significant extent. Whole body bioaccumulation factors measured for fish range from 17 to 90. Preference is given to results from flow through studies with concentration monitoring, such as Barrows et al. (1980). The bioconcentration factor predicted from K_{ow} using the equation in the TGD is 17.6. This value is in good agreement with the Barrows et al. (1980) and Korte and Freitag (1984) results, and will be used in the assessment of risk from secondary poisoning.

3.1.3 Aquatic compartment (including sediment)

Sediment will be considered in this section.

3.1.3.1 Calculation of Predicted Environmental Concentrations (PECs) in water

As described earlier, the major release of trichloroethylene is to the atmosphere and the release to the aquatic environment is likely to be small. The PECs of trichloroethylene in water have been calculated according to the methods in the TGD and the EUSES computer modelling program.

3.1.3.1.1 Local PECs for water

The local PECs for water have been calculated according to the TGD where full details on the calculations can be found.

As described earlier, biodegradation of trichloroethylene can occur under some circumstances, particularly in the presence of another substrate. However, trichloroethylene is not readily biodegradable according to the ready biodegradability tests. For this risk assessment, it has been assumed that trichloroethylene is not degradable. Using the SIMPLETREAT model as included in EUSES, with log K_{ow} of 2.29 and log H of 3, the removal of trichloroethylene in the WWTP is 92%. The fate of trichloroethylene in the WWTP is 91% is volatilised to air, 8% is dissolved in water and 1% is adsorbed to sludge.

The releases estimated in Section 3.1.1 for local sources have been used to calculate PEClocal for the water compartment based on release of trichloroethylene during use as an intermediate, handling (i.e. formulation as a solvent), metal degreasing and formulation and use in adhesives or consumer products. For these areas the default WWTP has been used. Concentrations have also been calculated for individual sites from information supplied by industry. The sites are referred to by a code number in the tables to maintain confidentiality. These cover all production sites and some sites where trichloroethylene is used as an intermediate. The PECs calculated are given in **Table 3.16**. The surface water calculations include the contribution from regional releases (see Section 3.1.3.1.2).

The $PEC_{microorganisms}$ is equivalent to the $Clocal_{eff}$, which is the concentration in the WWTP effluent before dilution. Values for $PEC_{microorganisms}$ have also been calculated and are in **Table 3.16**.

Table 3.16 Local PECs calculated for the aquatic environment

Process	Emission rate of trichloroethylene to wastewater (kg/day)	$PEClocal_{water}$ (µg/l) (including regional contribution)	$PEC_{microorganisms}$ (µg/l)
Site-specific (code)			
1A		19.4	150
1B		0.4	41
1C		0.45	0.8
3		0.38	0.08
4		0.65	no WWTP
5A		66	660
5B		0.4	0.3
Use as intermediate	6.7	28.4	281
Handling	0.25	1.4	11
Metal degreasing	4.6	19.6	193
Adhesives: formulation	1.0	4.5	42
Adhesives: use	0	0.35	0
Consumer products: formulation	0.46	2.3	19
Consumer products: use	0.06	0.62	2.7
Others	0.03	0.48	1.3

3.1.3.1.2 $PECregional_{water}$ and $PECcontinental_{water}$

The EUSES model has been used to predict regional and continental environmental concentrations of trichloroethylene in water, based on a tonnage of 138,000 tonnes per year in Europe. The $PECregional_{water}$ has been calculated on the basis of a standardised regional environment as described in the TGD. This regional environment is a densely populated area of 200·200 km with 20 million inhabitants. It has been assumed that the largest specific releases to air and to water occur within this area. The $PECcontinental_{water}$ has been calculated on the basis of the continental box which has the size of all EU countries together. The continental emission of trichloroethylene was set as 132,194 kg per day to air, 8,898 kg per day to wastewater, 3,814 kg/day direct to surface water and 138 kg/day to soil. The regional emission of

trichloroethylene was set as 17,400 kg per day to air, 1,003 kg per day to wastewater, 430 kg/day direct to surface water and 20 kg/day to soil.

Based on these estimates, the continental concentration of trichloroethylene in surface water was estimated to be 0.04 μg/l and the regional concentration was estimated to be 0.35 μg/l. (The EUSES output is in Appendix C).

3.1.3.2 Measured levels in water

3.1.3.2.1 Surface water

Levels of trichloroethylene in ocean waters, coastal and estuarine waters and other surface waters are given in **Tables 3.17**, **3.18** and **3.19** (where the reference is given as Rippen or BUA, the original references have not been checked for this assessment).

Table 3.17 Levels of trichloroethylene in ocean waters

Location	Concentration (μg/l)	Reference
Atlantic Ocean < 1977	0.0005-0.019	Selenka and Bauer (1977)
North Sea, 1983-4	< 0.005	Van de Meent et al. (1986)
Mediterranean, 1984	< 0.001	Marchand et al. (1988)
NE Atlantic ca. 1972	0.010	Atri (1985)
E Atlantic ca. 1972	0.005-0.0185	
South Pacific, 1981, 0-10 metres deep	0.0001-0.0007 mean, 0.0003	Singh et al. (1983)

Trichloroethylene has been monitored in surface waters of different regions of the UK (NRA, 1995). The results of this monitoring are given in **Table 3.20**.

McDonald et al. (1988) analysed samples from the Brazos River in the Gulf of Mexico which may be impacted on by shipping activities, industrial outfalls and urban runoff. Five cruises were carried out and trichloroethylene was detected during two of these cruises at levels up to 0.28 μg/l.

Table 3.18 Levels of trichloroethylene in coastal and estuarine water

Location and date	Concentration (μg/l)	Reference
North Sea	< 0.001-0.620	Rippen (1992)
North Sea Rhine estuary, 1983-4	< 0.005-0.026, mean 6 ng/l (108 measurements)	van de Meent et al. (1986)
North Sea, 1983	< 0.01-0.54	Hellmann (1984)
North Sea, British coasts, 1992	< 0.5	"
North Sea, German coast, 1983	< 0.01-0.54	"
Greece, coast, 1981-2	Thermiakos Gulf 0.26-2.30 Kavala Gulf 0.35 - 2.80	Fytianos et al. (1985)
Atlantic, Loire, 1983	0.063-0.182	Marchand et al. (1986)
Gulf of Mexico, 1977	Not detected (unpolluted) 0.010-0.050 (anthropogenic influence)	Sauer (1981)
Japan, 1985-7	0.6-19.7	Watanabe et al. (1987)

Table 3.19 Levels of trichloroethylene in surface waters

Location and date	Concentration (μg/l)	Reference
Germany		
Rhine, 1976	0.4-2.4, mean 1.1	Bauer (1981)
German rivers, 1980	<0.1 - 23.9	Rippen (1992)
" 1981	<0.1 - 2.0	
" 1982	<0.1 - 13.3	
" 1983	<0.1 - 8.2	
" 1984	<0.001 - 8.9	
" 1985	<0.001 - 79.9	
" 1986	0.001 - 14.4	
" 1987	0.001 - 4.2	
" 1988	<0.01 - 1.2	
" 1989	<0.001 - 0.77	
Rhine, Köln 1991 1992 1993	Mean 0.03, max 0.39 Mean <0.05, max 0.08 Mean <0.05, max 0.11	Umweltbundesamt, personal communication
Rhine tributaries, 1988-1989	< 0.01 - 0.75	BUA (1994)
Ruhr, 1988-1989	< 0.1 - 0.99	
Elbe, 1989	< 0.001 - 0.77	
Main, 1989	< 0.1 - 0.1	
Danube, 1989	< 0.1 - 1.0	
Weser, 1985-87	0.1	Bohlen et al. (1989)

Table 3.19 continued overleaf

Table 3.19 continued Levels of trichloroethylene in surface waters

Location and date	Concentration (μg/l)	Reference
Donau, 1985	< 0.4	van de Graaff (1986)
Main, 1985	average < 1	
Rhine, 1985	0.05 - 0.2	Hellmann (1984)
Main, 1982-3	max. 0.5	
Mosel, 1982-3	max. 0.25	
Neckar, 1981 and 1983	1981 0.4-0.8 1983 0.2-0.4	
Elbe, 1983	0.7-52.3	
France		
Loire, 1982-4	n.d. - 47	Marchand et al. (1986)
Loire estuary, 1982-4	0.05 - 0.182	
Rhone, 1984	0.059 - 0.100	Marchand et al. (1988)
Provence Côte d'azur, 1984	<0.001	
Nice, 1984	0.002-0.014	
Rhone, 1984	0.059-0.1	
Loire, 1983-4	0.009-0.113	
UK		
Bay of Liverpool (contaminated site)	3.6 (mean 0.30)	Pearson and McConnell (1975)
UK, lowland river, < 1981	< 0.8	Fielding et al. (1981)
Netherlands		
Rhine, 1990-1	< 0.1	RIWA (1991)
Rhine, 1986	0.2	Van der Graaff (1988)
Meuse, 1992 (Neth/Belgium)	0.04-0.2	RIWA (1992)
Finland, ca. 1981	0.04	Reunanen and Kronfeld (1982)
Sweden	0.002	Abrahamson and Klick (1989)
Switzerland		
River water, 1981-3	0.06	Fahrni (1984)
Zurich lake, < 1974	0.038 (surface) 0.065 (30 m depth)	Grob and Grob (1974)
USA, Susquehanna river, 1987	0.24	Smith (1989)

Dawes and Waldock (1994) collected water samples from estuaries and offshore sites around the UK and analysed them for trichloroethylene. Samples were taken at 0.2 m depth from the Tees, Tyne, Wear, Humber, Mersey and Tweed estuaries and found to contain up to 269 ng/l trichloroethylene. Samples from Poole, Southampton Water, the Firth of Forth, North Minch, Swansea Bay, the Bristol Channel, Queens Channel and Falmouth all contained less than 10 ng/l.

Dyksen and Hess (1982) have summarised results of monitoring data from studies in the USA. Trichloroethylene was detected in 28% of wells in eight States and the maximum surface water concentration reported was 160 μg/l.

Aggazzotti and Predieri (1986) surveyed various surface waters in the Emilia-Romagna district of Italy and found volatile halogenated organics in low amounts in more than half of the stations sampled. Trichloroethylene and tetrachloroethylene were the most represented compounds and the highest amounts were found just downstream of the most important towns or villages. An example of a contaminated station was a canal which collects wastes of the city of Modena where the mean trichloroethylene concentration was 32 μg/l.

Otson et al. (1982) carried out a survey of raw and treated potable water in Canada. Trichloroethylene occurred frequently in both raw and treated water. The average concentration in treated water was less than or equal to 1 μg/l but in winter raw water samples the average was 2 μg/l.

Table 3.20 Trichloroethylene levels in surface water in the UK

Location	Date	No. of samples	No. less than detection limit	Detection limit (μg/l)	Maximum (μg/l)
Region 1					
137 sites	1992-95	1,560	1,180	0.1-1.0	1,290
Region 2					
43 sites	1992	501	437	0.1-1.2	22.9
43 sites	1993	529	509	1.0-1.1	7.1
43 sites	1994	519	330	0.05-0.5	4.4
Region 3*					
2 sites	1992	53	51	1.0	0.1
12 sites	1993	55	54	1.0	0.6
12 sites	1994	63	61	1.0	0.5
73 sites	1992	261	247	1.0	41.65
73 sites	1993	283	271	1.0	98.0
73 sites	1994	289	277	1.0	86.5
Region 4					
40 sites	1992	803	Range 0.02-2.16		
40 sites	1993	507	Range < 0.02-2.11		
40 sites	1994	484	Range < 0.05-1.35		
Region 5					
113 sites Freshwater	1990-95	1,499	785	0.2	180
27 sites Estuary	1990-95	408	356	0.2	9.0
16 sites	1990-95	61	46	0.2	132
Region 6					
48 sites	1992-95	1,277	683	0.1	46.0
Region 7					
13 sites	1992-95	1,912	272	0.02-0.1	25.0

* different methods were used at different times so the detection limits varied between analyses. This is why some of the maximum values are less than the stated detection limit.

Wastewater levels

A survey of effluents of 114 industrial sites in the Rhone-Alpes region of France was carried out (INERIS, 1994). A single 24-hour mixing sample was taken at each site. Trichloroethylene was detected in 63 of the monitored effluents. The industrial activities undertaken at the various sites included the chemical industry, plastics and plastic fibres, solvent recycling, metalworking, textile dyeing, paint fabrication and surface treatment. Effluent concentrations up to 11,900 μg/l were found. Using the low flow rates in the receiving waters and summing releases to the same river in the same area gives concentrations up to 4.98 μg/l in the receiving water.

Municipal waters in Chester, Liverpool and Manchester (UK) were found to contain up to 0.6 μg/l trichloroethylene (Pearson and McConnell, 1975). US EPA (1981) included paint and ink formulation industries, electrical/electronic component manufacturers and rubber processing industries in those with mean effluent concentrations of trichloroethylene greater than 75 μg/l.

Fahrni (1984) measured trichloroethylene levels in 155 industrial water samples in Switzerland. Trichloroethylene was detected (> 0.1 μg/l) in 34% of samples and the average trichloroethylene concentration in these samples was 0.166 μg/l.

Lapp (1980) has measured trichloroethylene levels at six manufacturing sites and found levels upstream from the plant outlets between 0.4 and 353 μg/l and downstream from the plant outlet between 74 and 535 μg/l. Trichloroethylene levels upstream and downstream at the site of a user of trichloroethylene were 5 μg/l and 8-26 μg/l respectively. At 204 other sites in the USA, trichloroethylene concentrations were less than 6 μg/l at 95% of the sites and less than or equal to 1 μg/l at approximately 75% of the sites.

Measured levels of trichloroethylene in the range 0.06-0.23 mg/l in the wastewater of a European production site have been provided. A second company reported values of 0.07-0.08 μg/l. These values have been used in Section 3.1.3.1 to calculate the PEC for water at these sites.

3.1.3.2.2 Groundwater

Folkard et al. (1984) found trichloroethylene levels in groundwater in the UK between <0.1 and 38.5 μg/l. However, a report in ENDS (ENDS, 1989) gave results of two surveys of trichloroethylene levels in groundwater from industrial boreholes in Coventry and Birmingham which showed that highest trichloroethylene levels generally originated from premises involved in metal, electrical or rubber industries. In Coventry, 77% of 39 groundwater samples contained > 1 μg/l trichloroethylene and the maximum was 1,100 μg/l. The study of the Birmingham aquifer was carried out by Rivett et al. (1990) to assess the impact of a long established urban environment upon its underlying groundwater. The whole area of the aquifer is urbanised with 59% of the industrial land being used for metals/machinery. This aquifer is not used for public supply. Water samples were collected from 1986-1988 from 59 supply boreholes at depths between 20 and 300 m. Most boreholes were sampled on more than one occasion and trichloroethylene was detected (detection limit 0.02 μg/l) in 78% of the boreholes. In Birmingham, samples from 62% of 59 boreholes contained > 1 μg/l trichloroethylene with a maximum of 5,500 μg/l recorded.

CEFIC (1986) reported background levels for chlorinated solvents in groundwater of approximately 0.1 μg/l. Levels in Frankfurt in 1979 were found to range from 0.4-159 μg/l and in Mannheim in 1983 levels were between 30 and 130 μg/l. Average levels measured in the

Netherlands in 1982 ranged from < 0.01-1 μg/l. Groundwater from Italian cities had average trichloroethylene values between 0.1 and 1.1 μg/l and the maximum level was 158 μg/l.

Trichloroethylene has been found in a spring close to an industrial estate in the UK which drains into a stream (ENDS, 1995). Downstream of the spring the stream contained 273 μg/l trichloroethylene. At a drinking water abstraction, 15 km downstream, trichloroethylene was not detected.

Groundwater samples taken in Germany between 1977 and 1988 had maximum trichloroethylene levels of > 100 μg/l and minimum levels of < 0.001 μg/l and levels in other European countries ranged from 0.01 μg/l to 1,000 μg/l (Rippen, 1992).

Grob and Grob (1974) measured trichloroethylene in groundwater in Switzerland and found a concentration of 80 ng/l. Fahrni (1984) measured trichloroethylene concentrations in 92 groundwater samples from 46 sites in Switzerland and found 37% of samples contained measurable trichloroethylene (> 0.1 μg/l) and the average for these samples was 2.5 μg/l.

Nearly 2,000 groundwater samples were taken at various sites (489) in the UK and in 794 of these, trichloroethylene was below the detection limit (0.2 μg/l) (NRA, 1995). However, at some sites levels of trichloroethylene were extremely high. The two highest concentrations found were 950 mg/l and 10.9 mg/l.

A limited survey of eleven groundwaters used for public supply in the UK was carried out (Fielding et al., 1981). Trichloroethylene was not detected at only one site and levels were below 2 μg/l in eight other samples. High levels of 60 μg/l and 48 μg/l were found in raw water from two sites probably due to contamination of the aquifer.

Dyksen and Hess (1982) reported that the maximum groundwater concentration of trichloroethylene recorded in eight States in the USA was 35,000 μg/l. Westrick et al. (1984) found trichloroethylene to be one of the most frequent contaminants of USA groundwater in a survey of 446 randomly selected sites and 479 non-random sites.

He et al. (1992) carried out routine analysis of groundwater samples from three production wells and ten monitoring wells. The highest trichloroethylene levels in groundwater from a supply well and a monitoring well were 113 μg/l and 1,105 μg/l.

Trichloroethylene was found in leachate plumes of a sanitary landfill in Ontario, Canada (Reinhard et al., 1984). The trichloroethylene concentrations up to 2.9 μg/l were detected in three wells at depths of 19.6-25.6 m.

Levels of trichloroethylene in groundwater from other reports are summarised in **Table 3.21**. Estimated levels are provided in Section 3.1.4.1.

Table 3.21 Trichloroethylene levels in groundwater

Location and date	Average Concentration (μg/l)	Reference
Germany		
Regensburg and Barbing, 1985	< 1	Van der Graaff (1986)
Wells, 1984	< 0.01	Hellman (1984)
Nr Roth-Main river, 1985-1986	0.01	Herrmann (1987)
Waste disposal site, 1983-88	max. 0.8	Heil et al. (1989)
Berlin, 51 wells, < 1989	7-1500 (various pollution sources)	Leschber et al. (1990)
Nr River Main, 1987	27-80	Van der Graaff (1988)
Dusseldorf, 1983-5	0.9 (50% +ve)	Van der Graaff (1986)
987 water supplies, 1985-6	< 0.001-500	HOV-Studie (1987)
France, Val de Marne, 1983	10 m depth 0.9-30.2 40 m depth 2.6-22.5	Penverne and Montiel (1985)
England, 209 supply boreholes, 1984-5	35% > 1, 3 samples> 100	Folkhard (1986)
Austria, Vienna, 1982-3	100	Bolzer (1988)
Netherlands		
Ziest, 1987	up to 1000	Wilmanski and Van Breemen (1990)
Heemstede, 1983	< 0.01	Teekens (1985)
Italy		
Emilia-Romagna, 1981-3	3-12, mean 10.6	Aggazzotti and Predieri (1986)
Milan, 1982	12-80	Ziglio et al. (1983)
Milan, 1983	7	Anonymous (1986)
N. Italy, cities, 1981-2	0.1-1.1, max. 158	
3 cities, 1981-2	< 0.1-3.1	Ziglio et al. (1984)
Switzerland		
84 sites (North), 1981-3	0.92 (50% +ve)	Fahrni (1984)
Groundwater, < 1974	0.08	Grob and Grob (1974)
USA		
Groundwater systems, all sizes, < 1985	97% < 0.5 (estimated) 0.3% > 50 (estimated)	Cothern et al. (1986)
945 samples in 51 states, 1981-2	91+ve, 0.88-130	Westrick et al. (1984)
Edwards Aquifer and Cornal Springs, Texas, 1987	0.04 (detected in Cornal Springs sample only)	Buszka et al. (1990)
Nassau county, 1980	5.7-6.5	Connor (1984)
97 water supplies, Nebraska, 1984	< 0.2-26 (detected in 4 wells)	Goodenkauf and Atkinson (1986)
JAPAN, 1989	0.5-4300 (27% +ve)	Inamori et al. (1989)

In 1999 tetrachloroethylene was found in approximately 4% of samples taken in the general groundwater monitoring part of the general Danish Monitoring Programme NOVA (n ≈ 1,000),

whereas the detection frequency in drinking water wells was 10% (n ≈ 1,700) (Personal Communication (Danish EPA, 2001).

Summary

Trichloroethylene has been found at very high levels in groundwater. For most of the monitoring data available, there is no information about the source of the trichloroethylene in groundwater but it is likely that release is due to spillage or misuse. In recent years regulations aimed at protecting groundwater have been implemented in the EU. The effect of these regulations cannot be seen from the available measured data. There are not sufficient measurements, and the persistence of trichloroethylene at depth means that levels due to historical contamination will be evident for some time to come.

3.1.3.2.3 Other water samples

Drinking water

Levels of trichloroethylene in drinking water samples are given in **Table 3.22**.

Table 3.22 Levels of trichloroethylene in drinking water

Location and date	Average concentration (μg/l)	Reference
Germany		
100 cities, 1977	0.6 (range < 0.1-5.9)	Bauer (1981)
150 towns and cities, 1980	0.6-1.3	Von Düszeln and Thiemann (1985)
56 rural sites	0.1	
> 90% drinking water supplies, 1985	28.5%: 0.001-0.5 5.2%: 0.5-1.0 4.4%: 1.0-5.0 0.7%: 5.0-10.0 0.4%: 10-21	Ballschmiter et al. (1988)
120 cities, 1982	0.3	Ballschmiter et al. (1988)
Mannheim, 1988	5	Bächle (1990)
50 cities, 1980	0.6	Lahl et al. (1981)
Austria, Vienna, 1984	2.1 (max. 3.5)	Pfannhauser and Thaller (1985)
Finland, ca. 1981	0.42	Reunanen and Kronfeld (1982)
USA		
113 cities, ca. 1982	0.54 (max. 49.0)	Kraybill (1983)
22 locations, ca. 1980	Detected in 14 locations, ≤2 in 10 locations, max: 32	Lapp (1980)

A regular monitoring programme is in place for drinking water in England and Wales. This covers 31 areas and trichloroethylene levels were monitored at approximately 2,700 sites in 1994. The frequency of monitoring at each site depends on the size of the population supplied but the standard frequency is between four and twenty times per year. The vast majority of

measured trichloroethylene levels were below the detection limit (range 0.1-3.0 μg/l) although higher levels (up to 25 μg/l) have occasionally been detected.

Rainwater

Rainwater samples in the USA and an industrial area in England contained 0.78-16.5 ng/l and 150 ng/l respectively (Ligocki et al., 1985). Pearson and McConnell (1975) found trichloroethylene in rainwater samples in Runcorn at levels up to 0.15 μg/l. Snow samples from California and Alaska contained 1.5-30 ng/l and 39 ng/l of trichloroethylene respectively (Su and Goldberg, 1976). Rainwater samples from Germany, Switzerland and the UK had trichloroethylene levels of < 5-13,000 ng/l, < 11-1,000 ng/l and < 50 ng/l (Rippen, 1992). Samples of rainwater at 4 locations in the Netherlands contained less than 5 ng/l trichloroethylene although the authors have commented that these levels should be treated with care due to the inadequacy of laboratory procedures to produce proper blanks (van de Meent et al., 1986).

3.1.3.2.4 Summary of measured levels in water

In unpolluted surface waters (e.g. oceans), trichloroethylene concentrations are in the range 0.0001-0.02 μg/l and in other areas concentrations range from <0.0001 μg/l to approximately 10 μg/l. Other higher levels have been found but it is not known how representative these are. However, the vast majority of measurements are 1 μg/l or less. Groundwater levels are variable and subject to local contamination. In wide-ranging surveys, many concentrations are low, of the order of <0.2-2 μg/l. However, measurements in groundwater related to contaminated sites show high levels; up to 950 mg/l has been found. Levels of trichloroethylene in drinking water are generally less than 1 μg/l although some higher levels (up to 49 μg/l) have been reported.

3.1.3.3 Comparison of PEC with measured levels and selection of PEC values

A large number of measured concentrations are available for trichloroethylene in water, which show the range and variation in levels. The various calculated $PEClocal_{water}$ values of 0.14 μg/l to 66 μg/l for surface water are consistent with measured levels in surface water although PECs for production and use as an intermediate are at the highest end of the measured values. The $PECregional_{water}$ of 0.35 μg/l and $PECcontinental_{water}$ of 0.04 μg/l are also similar to measured values in surface water although levels in open ocean are lower than these predicted concentrations (higher levels have been found at contaminated sites but these are not comparable to regional or continental levels). The $PEClocal_{water}$ calculated from production of trichloroethylene at a typical site using emission factors from site-specific information (66 μg/l) will be used in the assessment. This is likely to be an overestimate as the calculation uses default values for WWTP size and river flow. A value of 10 μg/l will also be used as representative of the high end of measured values, the vast majority of reported values being below this value.

3.1.3.4 Sediment

3.1.3.4.1 Calculation of PECs for sediment

The values for $PEClocal_{water}$ can be used to calculate the $PEClocal_{sediment}$ using the methods in the TGD. The PECs calculated are given in **Table 3.23**. The EUSES model (Appendix C) has been used to calculate the regional and continental PECs for sediment and the results were 0.9 μg/kg and 0.1 μg/kg respectively.

Table 3.23 Local PECs calculated for sediment

Process	$PEClocal_{water}$ (μg/l)	$PEClocal_{sediment}$ (μg/kg)
Site-specific (code)		
1A	19.4	51
1B	0.4	1.1
1C	0.45	1.3
3	0.38	1.0
4	0.65	1.7
5A	66	181
5B	0.4	1.1
Use as intermediate	28.4	78
Handling	1.4	3.8
Metal degreasing	19.6	54
Adhesives: formulation	4.5	13
Adhesives: use	0.35	0.95
Consumer products: formulation	2.3	6.3
Consumer products: use	0.62	1.7
Others	0.48	1.3

3.1.3.4.2 Measured levels in sediment

Trichloroethylene was not detected in sediments in the vicinity of speciality chemical plants in the USA (Hites et al., 1979) but was detected in marine sediments at a maximum of 9.9 ng/g at Liverpool Bay in the UK (Pearson and McConnell, 1975). In the USA, trichloroethylene has been found in sediment samples at 6 km from the ocean outfall from the Los Angeles county wastewater treatment plant at <0.5 μg/kg (Gossett et al., 1983) and from Lake Pontchartrain, Louisiana, at levels between 0.1 and 0.2 μg/kg (Ferrario et al., 1985). Whelen et al. (1983) found trichloroethylene in an anoxic sediment from the Upper Basin of the Pettaquamscutt river, Rhode Island. Trichloroethylene was found in the 0-6 cm layer of sediment at 73 μg/kg and in the 6-12 cm layer at 65 μg/kg. Other levels of trichloroethylene in sediments are given in **Table 3.24**.

Table 3.24 Levels of trichloroethylene in sediments

Location and date	Average concentration (μg/kg)	Reference
Germany		
Rhine, 1982-3	9-13	BUA (1994)
Rhine, 1987-8	ca. 1-20	
Elbe, 1988	< 0.2-32	
Roth Main, 1985-6	0.2	Herrmann (1987)
Untermain, 1985, 3 sites	5-10	BUA (1994)
Neckar, 1984	2	
USA, 338 samples	< 0.5	Staples et al. (1985)

Walther et al. (1985) investigated trichloroethylene levels in sediment from a small catchment area used for agriculture and found a mean concentration of 26.4 μg/kg.

Samples of sludge from a wastewater treatment plant in Germany contained trichloroethylene at levels between 200 and 850 μg/kg in 1982 and between 15 and 240 μg/kg from 1972 to 1981 (Rippen, 1992). In the USA, sludge samples contained 0.048-44 μg/kg trichloroethylene in 1981 (Rippen, 1992).

Brown (1978) measured trichloroethylene levels in various sludges from sewage works in the UK which are a range of sizes and use different treatment processes and different types of crude sewage. At most works the trichloroethylene level in various sludges was in the range < 1 to 49 μg/kg. However, at two sites which receive industrial effluent, very high levels of trichloroethylene were found in settled sludge (up to 1,584 μg/l) and up to 76 μg/kg trichloroethylene was found in primary sludge.

Lapp (1980) found trichloroethylene in sediment from manufacturing and user sites up to 300 μg/kg.

3.1.3.4.3 Comparison of PEC for sediment with measured levels

The measured levels available for sediment are not as extensive as those for water although they do show a range of levels. The $PEClocal_{sediment}$ concentrations calculated for sediment range from 0.05 μg/kg to 181 μg/kg. The values are similar to the range of measured levels of trichloroethylene in sediments with one production site giving a value somewhat higher than those measured. The highest $PEClocal_{sediment}$ value calculated from site-specific information, 181 μg/kg, will be used in the assessment.

3.1.4 Terrestrial compartment

3.1.4.1 Calculation of PEC in soil

Three routes by which a substance can reach the terrestrial compartment are considered in this assessment: direct application, deposition from air and sewage sludge application. Direct releases of trichloroethylene to the terrestrial compartment are expected to be small; concentrations

resulting from the other two routes have been calculated using EUSES (Appendix C). The inputs from air and from sludge are derived from the emissions to air and water respectively, described in Section 3.1.1. The concentrations in the different soil types estimated are given in **Table 3.25**. Concentrations in groundwater under agricultural soil are also given. The percentage of steady state reached after ten years is 100%.

Table 3.25 Local PECs for soil and groundwater

Process	$PEClocal_{soil}$ (μg/kg wet weight)			$PEClocal_{grw}$ (μg/l)
	Agricultural soil (30 day average)	Agricultural soil (180 day average)	Grassland (180 day average)	
Production*	31	10.2	7.0	5.8
Use as intermediate	10	1.8	0.48	1.0
Handling	1.2	0.9	0.85	0.5
Metal degreasing	6.9	1.2	0.32	0.7
Adhesives: formulation	1.5	0.27	0.07	0.2
Adhesives: use	0.014	0.014	0.014	0.01
Consumer products: formulation	0.7	0.12	0.03	0.07
Consumer products: use	0.1	0.02	0.01	0.01
Other uses	0.05	0.01	0.004	0.006

* Production estimate is based on combination of largest individual release to air and to water from actual sites (not necessarily from the same site)

The EUSES model and parameters described earlier have been used to calculate the regional and continental concentrations in **Table 3.26**.

Table 3.26 PECregional and PECcontinental for soil

	$PECregional_{soil}$ (μg/kg w wt)	$PECcontinental_{soil}$ (μg/kg w wt)
Natural soil	0.002	0.001
Agricultural soil	0.008	0.001
Industrial soil	0.044	0.004
Pore water (μg/l)	0.005	0.001

3.1.4.2 Measured levels in soil

Soils from an industrial zone in Germany have been found to contain 3-4 μg/kg trichloroethylene (Rippen, 1992). Riverside soil from the Roth Main (1985-6) contained 0.06 μg/kg trichloroethylene (Herrmann, 1987). Trichloroethylene levels between <0.2 and 6.4 μg/kg were found in soil from Hamburg in 1984 (BUA, 1994).

Walther et al. (1985) measured trichloroethylene in soil samples from a small catchment area used for agriculture and found a mean concentration of 13 μg/kg.

US EPA (1977) have measured trichloroethylene levels in fourteen soil samples from the vicinity of production sites and found levels up to 0.62 μg/kg. Trichloroethylene levels in soil found at a site near a sewage plant in the USA were between 0.18 and 260 μg/kg (Rippen, 1992). The maximum trichloroethylene level found in USA soils (before 1980) was 5.6 μg/kg (Rippen, 1992).

Ellis et al. (1985) found trichloroethylene in the soil of ten US Superfund sites (out of 50 investigated) with maximum concentrations between 0.001 and 100 μg/kg. Trichloroethylene was also found in soil near two Superfund sites.

Frank et al. (1989) found levels of trichloroethylene in the soil-air of three sites in forests in Germany between 0.2 and 1.6 $\mu g/m^3$.

3.1.4.3 Comparison of PEC with measured levels

There are only a few measured levels of trichloroethylene in soil. Measured levels are typically lower than the local PECs calculated but higher than the regional and continental PECs calculated. Measured levels in groundwater (Section 3.1.3.2.2) are not directly comparable with the calculated values as they cannot be related to specific sources. The majority of the measured levels are lower than or similar to the calculated levels, but there are much higher measured levels in polluted areas. For this assessment, the $PEClocal_{soil}$ values from the production scenario (**Table 3.25**) will be used. These are 31 μg/kg for soil and 5.8 μg/l for groundwater.

3.1.5 Atmosphere

3.1.5.1 Calculation of PEC

The major release of trichloroethylene is to the atmosphere.

A PEClocal for air for release of trichloroethylene from production and various uses can be calculated. The following equation from the TGD can be used to calculate the concentration in air at 100 m from the site.

$$C_{air} = Emission \cdot Cstd_{air}$$

where:

C_{air} = concentration in air at 100 m from a point source (mg/m^3)
Emission = emission rate to air (kg/d)
$Cstd_{air}$ = standard concentration in air at source strength of 1 kg/s = $2.78 \cdot 10^{-4}$ mg/m^3

Various local PECs have been calculated and are given in **Table 3.27** (regional contribution is included).

Site-specific data from sites using trichloroethylene as an intermediate give calculated air concentrations ranging from background (0.43 $\mu g/m^3$) to 1.3 $\mu g/m^3$.

A PEClocal for air via emission from a wastewater treatment works can be calculated according to the equation from the TGD above. The largest release from a specific production site to a WWTP is 16.67 kg/day. This gives an air concentration of 4.2 $\mu g/m^3$.

Table 3.27 Local PECs calculated for the atmospheric environment

Process	Emission to air (kg/day)	$PEClocal_{air}$ (μg/m³)
Production - largest site-specific *	2,930	670
Use as intermediate	67	16
Handling	400	92
Metal degreasing	42	10
Adhesives: formulation	8.3	2.4
Adhesives: use	19	5.8
Consumer products: formulation	3.9	1.4
Consumer products: use	0.58	0.63
Others	0.29	0.55

* a measured concentration of 65 μg/m³ is available for this site, see Section 3.1.5.2.

The EUSES model and parameters described earlier have been used to calculate a PEC for the atmosphere on larger scales (Appendix C). The continental concentration of trichloroethylene in the atmosphere was estimated to be 0.18 μg/m^3 and the regional concentration was estimated to be 0.47 μg/m^3.

3.1.5.2 Measured levels in air

Measured levels of trichloroethylene in background, rural and urban air are given in **Tables 3.28**, **3.29** and **3.30**. Some of the urban air levels in **Table 3.30** may have been influenced by nearby industrial activity.

Table 3.28 Continental trichloroethylene levels

Location and date	Concentration (μg/m³)	Reference
Norwegian Arctic, 1983	0.04	Hov et al. (1984)
Norway, Spitzberg	0.05	Müller and Oehme (1990)
Southern hemisphere, 1989	0.003 (mean)	Koppman et al. (1993)
Northern hemisphere, 1989	0.016 (mean)	
Canadian Arctic, 1992-4	$5 \cdot 10^{-5}$ (summer) 0.044 (winter)	Yokouchi et al. (1996)
Alaska, 1982	0.03-0.076 (av. 0.06)	Rassmussen and Khalil (1983)
Troposphere, 1981/2	0.03	Pearson (1982)
Atlantic	< 0.017-0.05	Class and Ballschmitter (1986)

Table 3.28 continued overleaf

Table 3.28 continued Continental trichloroethylene levels

Location and date	Concentration (μg/m³)	Reference
Madeira, 1982	Pico Arieiro < 0.013 Porto Santo 0.076	Kirschmer and Ballschmiter (1983)
Azores, 1982	< 0.03	Kirschmer and Ballschmiter (1983)
Bermuda, 1985	< 0.02	Ballschmiter et al. (1988)
Eastern Pacific, 1981	North 0.066 South < 0.017	Singh et al. (1983)
Indian ocean, 1986	< 0.10	Ballschmiter et al. (1988)

Table 3.29 Trichloroethylene levels in rural air

Location and date	Concentration (μg/m³)	Reference
Germany: Asch , 1985	0.03-0.82	Hecht et al. (1987)
4 forest sites, 1986-88	0.45-0.55 (means)	Frank and Frank (1990)
Schwarzwald, 1986-8	0.1-3.5	Frank et al. (1990)
Berchtesgaden, 1989-90	0.02-0.68	
Garmisch, 1982	0.1-0.3	Selenka and Bauer (1984)
Alps, 1986	< 0.3-0.9	Bahadir et al. (1987)
Schwandorf, 1986	< 0.3-0.4	
Schwäbische Alb, 1985	Mean 0.38 (0.05-0.82)	Güthner et al. (1990)
Wank, 1984-8	2	Kubin et al. (1989)
general, 1988	< 0.2	Umweltbundesamt (1988)
Netherlands: 5 sites,1982-3	0.32-0.60	Thijsse and Huygen (1986)
Isle of Terschelling, 1980-1	mean 0.33	Guicherit and Schulting (1985)
Weiwerd	< 0.49	Correia et al. (1977)
Switzerland: ca. 1984	0.16	Fahrni (1985)
UK: rural areas, 1972	0.002-0.028	Murray and Riley (1973)
Forest of Dean	6	Pearson and McConnell (1975)
Canada: Walpole Island, Ontario, 1990	Mean 0.18, max 0.46	Dann and Wang (1992)
Japan, 1979-86	< 0.15	Makide et al. (1987)

Table 3.30 Trichloroethylene levels in urban and suburban air

Location	Concentration (μg/m³)	Reference
Germany		
Tübingen, 1988/89	0.4-16	Frank et al. (1991)
Hamburg, 1986/87	0.8-18.5 (means at 12 sites) 1.6 (overall mean)	Bruckmann et al. (1988)
Berlin, 1984/85	6.3-7.9	Seifert et al. (1986)
Frankfurt, Sep '83/Feb'84	1-10 (monthly means 1.8-6.3)	Müller and Riedel (1984)
Bremen, 1981/82	0.8 (range <0.1-16)	Von Düszeln and Thiemann (1985)
Bochum, 1978	<0.3-12.5	Bauer (1981)
Göppingen (small town), 1986	0.47-17	Hecht et al. (1987)
Munich, 1986	0.3-3.9	Bahadir et al. (1987)
Hof, 1985	< 0.7-10.8	Müller et al. (1986)
Munich	0.93-29.76	Correia et al. (1977)
Belgium		
Tessenderlo, 1988-9	1.8-2.3	Wauters and Verdun (1989)
Bruxelles (Nord)	3.7-5.6	Correia et al. (1977)
Bruxelles, 1974/75	6.0-31.7	Hecht et al. (1987)
France		
Paris, 1975	not detected - 4.1	Hecht et al. (1987)
Grenoble, 1975	6.8-28.9	
Lyon	<4.56-23.22	Correia et al. (977)
St Auban	0.93-44.8	
UK		
Moel Famau, Flintshire	1.2-10.8	Pearson and McConnell (1975)
Liverpool/Manchester suburban areas	1.2-24.1	
Industrial area (Runcorn)	48.2-77.1	
Widnes	10.8	Correia et al. (1977)
Liverpool, 1972-76	1.9-6.6	
Italy		
Turin, 1988	0.86-32.3, mean 8.36 (winter) 1.0-14.1, mean 3.14 (summer)	Gilli et al. (1990)
Milan	4-34	Ziglio (1981)
Italy, North, 1983-4	< 1-24	de Bortoli et al. (1986)
Sweden		
Stockholm, city centre	1.02-25.66	Jonsson et al. (1985)
Netherlands		
Hengelo	<0.11-0.93	Correia et al. (1977)
Three sites	0.32-0.75	Guicherit and Schulting (1985)
Delft	0.76	

Table 3.30 continued overleaf

Table 3.30 continued Trichloroethylene levels in urban and suburban air

Location	Concentration (μg/m³)	Reference
USA		
Portland, Oregon, 1984	0.24-3.9	Ligocki et al. (1985)
New Jersey, 3 sites, 1981-2	1.15-3.22	Harkov et al. (1984)
Houston, 1980	0.027-5.25	Singh et al. (1982)
St. Louis, 1980	0.043-5.58	
Denver, 1980	0.053-13.31	
Riverside, 1980	0.080-1.27	
Staten Island, 1981	0.139-5.39	
Pittsburgh, 1981	0.070-2.25	
Chicago, 1981	0.097-7.43	
San Jose, 1985	Mean 0.34 (0.044-1.45) –April Mean 0.37 (0.055-1.45) – August Mean 1.50 (0.39-4.95) - December	Singh et al. (1992)
Downey, 1984 (February)	Mean 1.0 (0.12-4.03)	
Houston, 1984 (March)	Mean 0.33 (<0.01-4.80)	
Denver, 1984 (March)	Mean 0.29 (0.027-1.32)	
Canada		
11 cities, 1988-1990	Means 0.07-0.45	Dann and Wang (1992)
Japan		
Nara city, 1986	0.76	Goto et al. (1987)
Hiroshima, 1984	< 0.3-0.9	Dohdoh et al. (1985)
Yokohama, industrial, 1985-6	8.2	Urano et al. (1988)
Kawasaki, commercial, 1985-6	5	
Nagoya, residential, 1985-6	3.7	

US EPA (1983) measured trichloroethylene concentrations in 10 towns in the USA and found levels from 0.03 to 16.52 μg/m^3 with average levels between 0.52 and 2.60 μg/m^3. A later survey of trichloroethylene levels in 6 towns in the USA (US EPA, 1986) found levels from <0.01 to 5.49 μg/m^3 with average levels between 0.29 and 1.46 μg/m^3. Bozzelli and Kebekkus (1982) measured trichloroethylene levels at residential and industrial locations in New Jersey. The average concentrations at the industrial sites were 9.8, 3.8, 6.6 and 7.6 μg/m^3; the residential sites had average concentrations of 1.3, 1.9, 1.1 and 1.1 μg/m^3. Harkov et al. (1984) found levels of trichloroethylene in New Jersey up to 67.2 μg/m^3. The mean trichloroethylene concentrations at three sites were between 1.15 and 3.23 μg/m^3. Lapp (1980) found trichloroethylene levels up to 16 μg/m^3 in 27 locations in the USA. Ambient levels at manufacturing sites were generally less than 13 μg/m^3 with the highest reported average being 76.4 μg/m^3.

More recent monitoring data from Germany show levels towards the lower end of the older data reported above. In Nordrhein-Westfalen in 1998, the highest annual mean level from around 40 sites was 0.88 μg/m^3, and the majority of the mean values were below 0.3 μg/m^3 (LUA, 1999).

In Japan, Hasegawa et al. (2000) reported an annual average air concentration of trichloroethylene of 3.5 μg/m^3 for three sites in a coastal industrial area.

Site-specific information from two European producers of trichloroethylene has been provided (personal communications, 1996 and 2001). Trichloroethylene levels measured in air at the site boundary of the first site ranged between 5 and 15 μg/m^3. At the second site, the mean of the daily average concentrations at the site boundary over a 28-day period was 65 μg/m^3.

Summary of measured levels in air

Measured background levels of trichloroethylene in unpolluted areas range from 0.003 to 0.1 μg/m^3. In rural areas trichloroethylene concentrations ranged from 0.002 to 6 μg/m^3 with most values less than 1. In urban and industrial areas, values typically range from 0.3 to approximately 30 μg/m^3, with mean values generally less than 10 μg/m^3. Higher levels have been found near industrial sites.

3.1.5.3 Comparison of PEC with measured levels

The PEC values calculated for the regional and continental compartments were 0.47 and 0.18 μg/m^3 respectively which are consistent with measured levels in rural air. The available measured concentrations show the range of levels of trichloroethylene in air. In urban and industrial areas, levels are typically 0.3-30 μg/m^3 and are consistent with the PECs calculated for the local environment. More recent measurements are found towards the lower end of this range. For this assessment, the calculated values for the various life cycle steps will be used, as they are similar to the higher measured values in industrial locations. However, for two production sites where measured concentrations are available the calculated concentrations, although based on monitored emissions, are higher than those measured, by a factor of six to ten times. The difference may be due to the distance to the site boundary in the real sites being different to that assumed in the model calculations. The monitoring data are considered to be valid, and so are used in preference to the calculated levels at these sites. As the higher measured value, 65 μg/m^3, is higher than the concentrations calculated for the other production sites, then this value will be used as a reasonable worst case for the air concentrations from production.

The calculated regional value will also be used in the assessment. In the risk assessment for dichloroacetic acid (Appendix B), the background data of Kloppman et al. (1993) are used in calculating trichloroethylene fluxes on the global scale.

3.1.6 Secondary poisoning

Indirect exposure of ecosystems may occur, particularly through the diffuse releases of trichloroethylene. Trichloroethylene is not biodegradable, but it does not bioaccumulate to a significant extent. Classification of trichloroethylene with risk phrases R45 and R68 is appropriate. Therefore a risk characterisation for secondary poisoning will be carried out.

For the assessment of secondary poisoning, concentrations in fish and earthworms are calculated from the estimated concentrations in water and soil. For fish a bioconcentration factor of 17.6 is used (as discussed in Section 3.1.2.4). For earthworms, the bioconcentration factor predicted from the log Kow value according to the TGD is 4.43. It is assumed that for predatory animals, 50% of their diet comes from the local area, and 50% from the region. Thus the concentrations required for the assessment are the averages of the local and regional concentrations in fish and

worms. These derived concentrations are presented in **Table 3.31**. Note that only the highest concentration in fish or worms from the individual production site results has been included in the table. Measured concentrations in fish are reported in Section 3.1.7.2. Overall, the levels measured in fish cover the range from 1 μg/kg to 350 μg/kg for whole fish; the predictions below cover a similar range of values.

Table 3.31 Predicted concentrations in fish and worms for secondary poisoning assessment

Process	Concentration (μg/kg)	
	Fish	Worms
Production	513	23
Use as intermediate	210	4.0
Handling	14	2.0
Metal degreasing	146	2.7
Adhesives: formulation	37	0.6
Adhesives: use	6.1	0.05
Consumer products: formulation	20	0.29
Consumer products: use	8.5	0.06
Others	6.5	0.04

3.1.7 Humans exposed via the environment

The EUSES program (Appendix C) has been used to calculate the potential human intake of trichloroethylene on the regional scale, from exposure to background concentrations (Section 3.1.7.1 below). The program uses the methods described in the TGD to estimate the concentrations of trichloroethylene in foodstuffs (plants, milk and meat) from the calculated concentrations in air, water and soil. Standard daily intake values are then used to estimate the daily dose.

For exposure on the local scale, concentrations measured in food and water have been used in preference to the calculated values. The measured data are presented in Section 3.1.7.2, and the resulting uptake is calculated in Section 3.1.7.3.

3.1.7.1 Predicted human intakes at the regional level

The daily human intake of trichloroethylene has been estimated by EUSES (Appendix C). The intakes are presented in the third column of **Table 3.32**. The estimation is based upon typical human consumption and inhalation rates presented in the second column. Total intake is calculated to be $1.4 \cdot 10^{-4}$ mg/kg/day.

Table 3.32 Regional concentrations in air, water and biota and the calculated human intake

Concentrations in air, water and biota intake	Human consumption or intake rate per day	Human intake via indirect exposure (mg/kg/day) [a,b]
Air = 0.47 μg/m^3	20 m^3	Air = $1.3 \cdot 10^{-4}$
Drinking water = 0.17 μg/l	0.002 m^3	Drinking water = $5.0 \cdot 10^{-6}$
Fish = 6.1 μg/kg	0.115 kg	Fish = $1.0 \cdot 10^{-5}$
Leaf/Stem crop = 0.004 μg/kg	1.2 kg	Stem of plants = $6 \cdot 10^{-8}$
Root crop = 0.015 μg/kg	0.384 kg	Root of plants = $8 \cdot 10^{-8}$
Meat = 3 x 10^{-4} μg/kg	0.301 kg	Meat = $1.4 \cdot 10^{-9}$
Milk = 5 x10^{-4} μg/kg	0.561 kg	Milk = $4 \cdot 10^{-9}$
		Total = $1.5 \cdot 10^{-4}$

a) Assuming a 70 kg person.
b) Assuming an intake of 100%.

3.1.7.2 Measured levels in biota and foodstuffs

3.1.7.2.1 Biota

Pearson and McConnell (1975) found trichloroethylene in tissues of aquatic invertebrates and fish and in the eggs of sea and freshwater birds. Trichloroethylene levels found in aquatic invertebrates and fish are given in **Table 3.33**. All organisms were collected from Liverpool Bay, Thames Estuary, Tees Bay, Firth of Forth, Mersey Estuary and Torbay. The results reported here are the levels in flesh. Levels in the liver were also measured for some fish and were typically higher than levels in flesh.

Dickson and Riley (1976) also measured levels in aquatic invertebrates and fish sampled from the Irish Sea. These levels were typically higher than those measured by Pearson and McConnell (1975) with the highest level recorded in the liver of the dogfish (*Scylliorhinus canicula*) of 479 μg/kg dry weight.

Pearson and McConnell (1975) found trichloroethylene in eggs and tissues of birds and the highest concentration was 33 μg/kg, found in eggs of the Kittiwake (*Rissa tridactyla*). Grey seals (*Halichoerus grypus*) sampled on the Farne Islands were also found to contain trichloroethylene (2.5-7.2 μg/kg) and levels in the common shrew (*Sorex araneus*) sampled at Frodsham Marsh in Merseyside were between 2.6 and 7.8 μg/kg.

Ofstad et al. (1981) measured levels of trichloroethylene in various fish from regions with different industries. A summary of these levels is given in **Table 3.34**.

Table 3.33 Levels of trichloroethylene in aquatic invertebrates and fish (Pearson and McConnell, 1975)

Organism	Level (μg/kg wet weight)
Plankton	0.05-0.4
Mussel (*Mytilus edulis*)	4-11.9
Oyster (*Ostrea edulis*)	2
Cockle (*Cerastoderma edule*)	6-11
Slipper limpet (*Crepidula fornicata*)	9
Crab (*Cancer pagurus*)	2.6-15
Shore crab (*Carcinus maenus*)	12
Hermit crab (*Eupagurus bernhardus*)	5-15
Shrimp (*Crangon crangon*)	16
Marine algae (Mersey estuary)	16-23
Ray (*Raja clavata*)	0.8-5
Plaice (*Pleuronectes platessa*)	0.8-8
Flounder (*Platycthys flesus*)	3
Dab (*Limanda limanda*)	3-5
Mackerel (*Scomber scombrus*)	5
Sole (*Solea solea*)	2
Red gurnard (*Aspitrigla cuculus*)	11
Scad (*Trachurus trachurus*)	2
Spurdog (*Squalus acanthias*)	3
Spratt (*Clupea sprattus)*	3.4
Cod (*Gadus morhua*)	0.8
Pout (*Trisopterus lscus*)	2

Table 3.34 Levels of trichloroethylene in fish (Ofstad et al., 1981)

Sample	Location and Date	Trichloroethylene level (μg/kg)	Industry
Sprat	Frierfjord, 1975	210	Chemical plant
"	"	75	"
Cod fillet	Frierfjord, 1981	not detected	"
Cod liver	"	250	"
Cod liver	"	400	"
Eel	Iddefjord, 1980	300	Pulp and paper factory
"	"	350	"
Whiting	"	50	"
"	"	30	"
Perch	Lake Mjosa, 1980	340	Pulp and paper factory
Roach	"	250	"
Cod	Kristiansand, 1980	not detected	Various
Salmon fillet	River Otra	not detected	Pulp and paper factory
Freshwater herring	Lake Tyriffjord, 1980	not detected	Various, agriculture, landfill
Trout fillet	"	not detected	"

Trichloroethylene levels were measured in fish from the Alzkanal in Germany in 1985 (van de Graaff, 1986). Trichloroethylene levels were measured in the gills, muscle, liver, spleen, kidney and gonad of fish and the highest concentration found was 80 ng/g wet weight in the spleen.

Further levels of trichloroethylene in biota are given in **Table 3.35** (the original references marked * have not been checked for this assessment).

Table 3.35 Levels of trichloroethylene in biota

Species, location and date	Mean concentration (μg/kg wet weight)	Reference
Oysters and mussels, Lake Pontchartrain, USA, 1980	0.8-5.7	Ferrario et al. (1985) *
Invertebrates, Palos Verdes, USA, 1980-1	7 shrimp muscle 0.3	Gossett et al. (1983)
Various species, Norway, 1980	0.5-1.8 (pure water) 0.5-96 (contaminated water)	Baumann-Ofstad et al. (1981) *
Anguilla anguilla, 1988 Germany, settling pond	54	Diezel et al. (1988) *
Fish tissues, Germany, Donau, 1985	0-10	van de Graaff (1986)
Fish tissues, Germany, Alzkanal, 1985	0-51, upper part 2-80, lower part	
Various fish, Germany, Rhine, 1981	< 1	Binnermann (1983) *

Overall, the levels measured in fish cover the range from 1 μg/kg to 350 μg/kg for whole fish; the levels predicted in Section 3.1.6 cover a similar range of values.

3.1.7.2.2 Foodstuffs

Levels of trichloroethylene in food are given in **Table 3.36** (where references are marked *, the original references have not been checked for this assessment).

Table 3.36 Trichloroethylene levels in food

Sample, location and date	Concentration (μg/kg)	Reference
Dairy products		
Butter, Germany, 1977-8	1.1	Bauer (1981)
Butter, UK, 1975	10	McConnell et al. (1975)
Butter, Germany, 1982	< 0.3	Selenka and Bauer (1984) *
Butter, USA, 1987	1.6-20	Heikes (1987)
Eggs, UK, 1975	0.6	McConnell et al. (1975)
Eggs, Germany, 1982	max. 567, mean 74	Selenka and Bauer (1984) *
Eggs, Germany, 1983	n.d.-26, mean 7.6	UBA-Bericht (1983) *
Milk, UK, 1975	0.3	McConnell et al. (1975)
Milk, Finland, 1987	0.1 ± 0.06	Kroneld (1989)
Milk prods. with fruit, Germany, 1978	max. 3.1, mean 0.7	Bauer (1981)
Milk, Germany, 1977-8	0.1-0.7, mean 0.2	
Buttermilk, Germany, 1977-8	< 0.1	Bauer (1981)
Yogurt, Germany, 1977-8	< 0.1-1.2	
Cheese, Germany, 1977-8	0.5	"
Cheese, UK, 1975	3	McConnell et al. (1975)
Cheese, USA, 1987	n.d.-10	Heikes (1987)
Oils and fats		
Olive oil, Germany, 1977-8	0.5-4.7, mean 2	Bauer (1981)
Olive oil, UK, 1975	9	McConnell et al. (1975)
Margarine, Germany, 1977-8	0.3	Bauer (1981)
Margarine, UK, 1975	6	McConnell et al. (1975)
Margarine, USA, 1987	3.7-980	Heikes (1987)
Cod liver oil, Germany, 1977-8	3	Bauer (1981)
Cod liver oil, UK, 1975	19	McConnell et al. (1975)
Vegetable oil, UK, 1975	7	

Table 3.36 continued overleaf

Table 3.36 continued Trichloroethylene levels in food

Sample, location and date	Concentration (μg/kg)	Reference
Drinks		
Decaffeinated coffee, Germany, 1983	n.d.-150, mean (51.7)	UBA-Bericht (1983) *
Freeze-dried coffee, UK, 1975	4	McConnell et al. (1975)
Coffee, Finland, 1987	1.2 ± 0.09	Kroneld (1989)
Tea in bags, UK, 1975	60	
Soft drinks, Germany, 1977-8	max. 11.6, mean 1.9	Bauer (1981)
Soft drinks, Germany, 1982	1.1	Selenka and Bauer (1984) *
Fruit drink, UK, 1975	5	McConnell et al. (1975)
Light beer, UK, 1975	0.7	
Wine, UK, 1975	0.02	
Cereals		
Fresh bread, UK, 1975	7	McConnell et al. (1975)
Various breads, flour and starchy foods, Germany, 1977-8	max. 4	Bauer (1981)
Various ready to eat cereal products	n.d. - 9.2	Heikes (1987)
Meat		
Various meat products, Germany, 1977-8	< 0.6-16	Bauer (1981)
Pork, Germany, 1983	n.d.-586 (mean 18.5)	UBA-Bericht (1983) *
Sausage, Germany, 1978	max. 192, mean 23.4	Bauer (1981)
Sausage, Germany, 1982	2	Selenka and Bauer (1984) *
Meat, UK, 1975	16-22	McConnell et al. (1975)
Meat, Finland, 1987	0.5 ± 0.01	Kroneld (1989)
Others		
Fish, Finland, 1987	0.1 ± 0.06	
Fruit and vegetables, UK, 1975	u p to 5	McConnell et al. (1975)
Fruit and vegetables, Germany, 1977-8	< 0.6-6	Bauer (1981)

Trichloroethylene has also been found in various foodstuffs. McConnell et al. (1975) found up to 60 μg/kg trichloroethylene in tea and up to 1.7 μg/kg in fruit and vegetables. Pearson (1982) measured levels up to 10 μg/kg in dairy produce, 12-22 μg/kg in meat, up to 9 μg/kg in vegetable oils and 7 μg/kg in bread. Very high levels of trichloroethylene were found in margarine samples from two areas of the USA with average levels of 440 and 3,600 μg/kg.

Heikes and Hopper (1986) used a purge and trap method for determining trichloroethylene concentrations in whole grains, milled grain products and intermediate grain based foods. The recoveries of trichloroethylene from these foods fortified with trichloroethylene ranged from 86-100%. Trichloroethylene was not detected (detection limit 0.5 ng/g) in wheat (nine samples), corn (two samples), oats, corn meal (two samples), corn grits, corn muffin mix, dried lima beans, lasagna noodles, a sample of bleached flour and uncooked rice. However, trichloroethylene was detected in yellow corn meal (2.7 ng/g), fudge brownie mix (2.3 and 2.5 ng/g), bleached flour (0.77 ng/g) and yellow cake mix (1.3 ng/g).

Daft (1989) analysed 549 food samples and found fumigant residues in 372 of these. Trichloroethylene was detected at levels between 2 and 94 ng/g in five of these samples and the mean trichloroethylene level was 49 ng/g.

Trichloroethylene has also been detected in cough mixtures (Bauer, 1981). Seven cough mixtures were analysed and trichloroethylene was found in three of these at levels up to 3.4 μg/l.

3.1.7.3 Local human exposures via environmental routes

The data presented in column 1 in **Table 3.37** below have been used together with the daily intake presented in column 2 to calculate the human intake via indirect exposure. The sources of the data in column 1 are referenced. They represent worst-case values from the measured levels available, with the exception of the air level which is the highest calculated air level (annual average) for a site with specific release data.

In addition, EUSES was used to calculate local human exposure via the environment for each of the scenarios described in Section 3.1.1. These all gave values which were lower than that calculated below.

Table 3.37 Local concentrations in air, water and food and the calculated human intake.

Concentration in air, water and biota [a)]	Daily human consumption or intake	Calculated human intake via indirect exposure ($mg \cdot kg^{-1} \cdot day^{-1}$) [b,c)]
Air = 65 μg/m³ ($PEClocal_{air}$ measured near a production plant)	20 m³	0.019
Drinking water = 21 μg/l (Ballschmiter et al., 1988, worst case)	0.002 m³	$6 \cdot 10^{-4}$
Fish = 479 μg/kg (Dickson and Riley, 1976, dogfish)	0.115 kg	$7.9 \cdot 10^{-4}$
Leaf/stem crop = 6μg/kg (Bauer, 1981)	1.2 kg	$1.0 \cdot 10^{-4}$
Root crop = 6 μg/kg (Bauer, 1981)	0.384 kg	$3.3 \cdot 10^{-5}$
Meat = 192 μg/kg (Bauer, 1981)	0.301 kg	$8.3 \cdot 10^{-4}$
Milk/dairy products = 20 μg/kg (Heikes, 1987)	0.561 kg	$1.6 \cdot 10^{-4}$
		Total = 0.022 $mg \cdot kg^{-1} \cdot day^{-1}$

a) References from Sections 3.1.7.2.1 and 3.1.7.2.2 above.
b) Assuming a 70 kg person.
c) Assuming an intake of 100%.

3.1.8 Summary of PEC values

The PEC values in **Table 3.38** will be used in the risk characterisation, in addition to those calculated for individual life cycle stages.

Table 3.38 Summary of PEC values

Area	PEC value
Local	Air (site-specific calculation) = 65 μg/m^3 Surface water (site-specific calculation) = 66 μg/l Surface water (measured, representative) = 10 μg/l Agricultural soil (calculated) = 31 μg/kg Sediment (site-specific calculation) = 181 μg/kg
Regional	Air = 0.47 μg/m^3 Surface water = 0.35 μg/l Agricultural soil = 0.008 μg/kg Sediment = 0.9 μg/kg
Continental	Air = 0.18 μg/m^3 Surface water = 0.04 μg/l Agricultural soil = 0.001 μg/kg Sediment = 0.1 μg/kg

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

3.2.1 Aquatic compartment (including sediment)

In discussing aquatic toxicity studies, it should be noted that the volatility of trichloroethylene makes it difficult to maintain the exposure concentrations. Test results have therefore to be interpreted with caution. Appendix D describes the studies in more detail where available, and indicates their validity.

3.2.1.1 Toxicity to microorganisms

Acute toxicity testing has been carried out on a variety of aquatic species and some chronic toxicity tests have been carried out. Results of some toxicity tests of trichloroethylene to microorganisms are shown in **Table 3.39**.

Belay and Daniels (1987) investigated the effect of trichloroethylene on the growth of methanogens. Cultures were incubated with trichloroethylene in the dark with shaking; duplicate tests were carried out at each concentration. A trichloroethylene concentration of 17.7 mg/l was found to inhibit the growth of *Methanobacterium thermoautotrophicum* and 70.7 mg/l inhibited the growth of *Methanococcus thermolithotrophicus*.

No information is available on the effects of trichloroethylene on organisms in groundwater.

3.2.1.2 Toxicity to aquatic plants

Various tests have been carried out to study the toxicity of trichloroethylene to aquatic plants. A summary of the conditions used and the results is shown in **Table 3.40**.

Erickson and Hawkins (1980) determined a 25% increase in photosynthesis (based on ^{14}C-CO_2 uptake) in a mixed population of green algae and blue algae after exposure to trichloroethylene for 24 hours in a flow through system. Trichloroethylene stimulated ^{14}C uptake at 0.5-1.0 mg/l but at higher concentrations there was no significant difference between the exposed population and the controls.

Biggs et al. (1979) investigated the inhibition of algal growth in the species *Dunaliella tertiolecta* and *Thalassiosira guillardii* by exposure to trichloroethylene at concentrations of 50 or 100 μg/l for 72 hours. No inhibition of growth was observed at either concentration.

Table 3.39 Toxicity of trichloroethylene to microorganisms

Species	Conditions	Result	Reference
Nitrosomonas sp.	24 hour, 25°C, pH 6.5-8, static, sealed, questionably accurate results	EC_{50} 0.81 mg/l	Blum and Speece (1991a)
Photobacterium phosphoreum	5 minutes, Microtox test	IC_{50} 960 mg/l	Blum and Speece (1991b)
	10 minutes	EC_{50} 602 mg/l	Bazin et al. (1987)
	15 minutes, Microtox test	EC_{10} 75 mg/l EC_{50} 115 mg/l	De Zwart and Slooff (1983)
	5 minutes, Microtox test	EC_{10} 87 mg/l EC_{50} 156 mg/l	"
	15 minutes	EC_{50} 190 mg/l	Hermens et al. (1985a)
Pseudomonas putida	9 hour, cell doubling time, 21°C	Control 1.5 hrs Exposed 5 hrs	Wackett and Householder (1989)
	16 hours, static, 25°C, inhibition of cell multiplication, nominal concentration	Toxicity threshold 65 mg/l	Bringmann and Kühn (1980a)
	30 minutes, ring test, 7 labs, 20°C	Toxic tolerance limit 547 mg/l	Robra (1979)
	16 hour, ring test, 11 labs	Toxicity threshold 81mg/l	Trenel (1972)
Aerobic heterotroph	24 hour, inhibition of oxygen consumption, pH 7, 25°C	IC_{50} 130 mg/l	Blum and Speece (1991a)
Heterotrophic bacteria	24 hour, 20°C, change in bacterial degradation	EC_{10} 63 mg/l EC_{50} 160 mg/l	Krebs (1985)
Other bacteria (methanogens)	24 hour, gas production, pH 7, 35°C	IC_{50} 13 mg/l	Blum and Speece (1991a)
Chilomonas paramaecium	48 hour	EC_3 >400 mg/l	Bringmann and Kühn (1981)
Entosiphon sulcatum	Static, 72 hours, 25°C, inhibition of cell multiplication test, nominal concentration	Toxicity threshold 1,200 mg/l	Bringmann and Kühn (1980a)
Tetrahymena pyriformis	24 hour, static, growth inhibition, 30°C	EC_{50} 410 mg/l	Yoshioka et al. (1985)
Uronema parduzci	20 hour, inhibition of cell multiplication, 25°C, static, nominal concentration	EC_3 >960 mg/l	Bringmann and Kühn (1980b)
Activated sludge	OECD guideline 209, activated sludge respiration inhibition test, sealed, static	EC_{50} 260 mg/l	Volskay and Grady (1986)

Tadros et al. (1994) investigated the response of various green algal species to trichloroethylene. All test organisms were assayed in water-soluble fraction concentrations of 0.05, 0.1 and 0.2 ml added to 100 ml growth medium (rapporteur's note: this is assumed to mean adding 0.05, 0.1 or 0.2 ml of a saturated solution of trichloroethylene to the medium. As such, the actual concentrations in the exposures are not defined). Each assay was conducted in triplicate in test tubes inoculated from exponential growing cells at an initial density of $4 \cdot 10^3$ cells/ml. All cultures were incubated on a shaker for 96 hours at a temperature of 30°C. Trichloroethylene at a

concentration of 0.05% (by weight) stimulated the growth of *Gleocystis ampla* to almost double that in the control. At higher concentrations the response was less but still positive. *Nannochloris* sp. and *Tetraselmis* sp. were not inhibited by trichloroethylene at all concentrations while *Scenedesmus obliquus*, *Chlorella ellipsoidea* and *Chlorococcum* sp. were progressively inhibited with increasing trichloroethylene concentrations.

Table 3.40 Toxicity of trichloroethylene to aquatic plants

Species	Conditions	Result	Reference
Chlamydomonas reinhardii	72 hour, growth measured by chlorophyll content, sealed vessels	EC_{10} 12.3 mg/l EC_{50} 36.5 mg/l	Brack and Rottler (1994)
Microcystis aeruginosa	8 days, growth rate	Toxicity threshold 63 mg/l (=EC_3)	Bringmann and Kühn (1978)
Phaeodactylum tricornutum	Photosynthesis	EC_{50} 8 mg/l	Pearson and McConnell (1975)
Scenedesmus quadricauda	8 day, growth rate	Toxicity threshold >1,000 mg/l (=EC_3)	Bringmann and Kühn (1978)
Scenedesmus sp.	96 hour, growth rate, static, 22°C (test not suitable for volatile compounds)	EC_{10} 300 mg/l EC_{50} 450 mg/l	Geyer et al. (1985)
Scenedesmus subspicatus	24 hour, population growth test	EC_{10} 70-82 mg/l	Scheubel (1984)
	96 hour, inhibition of cell multiplication. Gas-tight stoppers used.	EC_{10} 46-61 mg/l	Scheubel (1984)
Selenastrum capricornutum	96 hour, growth rate, 26°C, nominal concentration	NOEC 175 mg/l	Slooff et al. (1983)
Skeletonema costatum	96 hour, static, 20°C, salinity 30 ppt, based on initial concentration	EC_{50} 150 mg/l	Ward et al. (1986)
Mixed culture	24 hour, change in photosynthesis output	EC_{10} 230 mg/l EC_{50} 530 mg/l	Krebs (1985)

Tadros et al. (1995) collected and isolated seven species of diatoms from the intertidal region of the Gulf of Mexico. These species were maintained and tested in a medium enriched with artificial sea salt mix, and with trace elements, with a salinity of 20 ppt and a pH of 8.0 to 8.2. All test organisms were assayed in triplicate in water-soluble fraction concentrations of 0.2, 0.25 and 0.3 ml added to 100 ml medium (see note above). Cultures of exponentially growing cells at an initial density of approximately $4 \cdot 10^3$ cells/ml were incubated on a shaker for 96 hours at 30°C under cool white light. Growth was then measured spectrophotometrically. *Nitzschia dissipata* sp. and *Thalassiosira weissflogii* sp. were stimulated at 0.2% trichloroethylene. *Cyclotella* sp., *Cyclindrotheca* sp., *Nitzschia* sp., and *Navicula saprophila* sp. all tolerated the chemical but *Nitzschia pussilla* sp. was sensitive to trichloroethylene at all concentrations.

3.2.1.3 Toxicity to aquatic invertebrates

3.2.1.3.1 Acute toxicity

Several tests on the acute toxicity of trichloroethylene towards aquatic invertebrates have been carried out, and the results and conditions of these tests are summarised in **Table 3.41**.

Table 3.41 Results of acute toxicity tests of trichloroethylene on aquatic invertebrates

Species	Conditions	Result	Reference
Water flea *Daphnia cucullata*	Static, 48 hour, age 10-12 days, nominal conc.	EC_{50} = 56-58 mg/l	Canton and Adema (1978)
Water flea *Daphnia magna*	Static, closed, 48 hour, age <1 day, Dutch std. method Concept NEN 6501	IC_{50} 20.8 mg/l	Hermens et al. (1984)
	24 hour, Standard AFNOR	EC_{50} = 76 mg/l	Bazin et al. (1987)
	Static, 48 hour, EPA-660/3-75-009, age <24 hour, 21-23°C, nominal conc., pH 7.4-9.4, hardness 173 mg/l $CaCO_3$, open containers	EC_{50} = 18 mg/l, NOEC = 2.2 mg/l	Leblanc (1980)
	Static, 48 hour, EPA-660/3-75-009, 20±1°C, dechlorinated lake water, open containers, nominal concentrations	LC_{50} = 2.2 mg/l	McCarty (1979)
	Static, sealed, 48 hour, age 4-6 days, 21-25°C	EC_{50} = 7.8 mg/l	Abernethy et al. (1986)
	Static, 48 hour, age <24 hour, nominal concentration	EC_{50} = 42-97 mg/l	Canton and Adema (1978)
	Static, 48 hour, nominal concentration	EC_{50} = 85.2 mg/l	US EPA (1980)
	Swim test	EC_{50} = 27 mg/l	Korte and Greim (1981)
Water flea *Daphnia pulex*	Static, 48 hour, age <72 hours, 20±1°C	EC_{50} = 39-51 mg/l	Canton and Adema (1978)
Mysid shrimp *Mysidopsis bahia*	96 hour, static, closed glass dishes, measured concentration (av. initial and final)	EC_{50} = 14 mg/l	Ward et al. (1986)
Water flea *Moina macropoda*	3 hour	EC_{50} = 200 mg/l	Yoshioka et al. (1986)

The study by McCarty (1979) reports only nominal concentrations and as open test vessels were used the exposure concentrations cannot be defined. In the study by Abernethy et al. (1986) the exposure solutions were made up from a saturated solution of trichloroethylene. The concentration in such a saturated solution may vary according to the conditions under which it is made; as the specific concentration in this case was not analysed then the actual exposure concentrations cannot be determined. Additionally, *Daphnia* were cultured and exposed in distilled water, which would have put the organisms under stress. Neither of these two studies is therefore considered valid.

Slooff (1983) has measured 48-hour EC_{50} values for various aquatic invertebrate species and these are given in **Table 3.44**. The tests were carried out in Dutch Standard Water in a closed, static system at 20±1°C. The actual trichloroethylene concentrations were not analysed so the EC_{50} values were calculated using nominal concentrations.

Slooff et al. (1983) have also measured 48-hour LC_{50} values for third instar lifestage of the insects *Aedes aegypti* and *Culex pipiens* (both mosquito species). The test medium used was Dutch standard water at 26°C. The LC_{50} values, based on nominal concentrations, were 48 mg/l for *A. aegypti* and 55 mg/l for *C. pipiens*.

Table 3.42 Acute toxicity of trichloroethylene to aquatic invertebrates

Species	EC_{50} value
Freshwater shrimp (*Gammarus pulex*)	24 mg/l
Isopod (*Asellus aquaticus*)	30 mg/l
Water boatman (*Corixa punctata*)	110 mg/l
Dragon fly (*Ischnura elegano*)	49 mg/l
May fly (*Cloeon dipterum*)	42 mg/l
Stonefly (*Nemona cinerea*)	70 mg/l
Midge (*Chironomus thummi*)	64 mg/l
Gastropod (*Lymnaea stagnalis*)	56 mg/l
Tricladid (*Dugesia lugubris*)	42 mg/l
Aquatic worm (*Erpobdella octoculata*)	75 mg/l
Aquatic worm (oligochaete family)	132 mg/l
Coelenterate (*Hydra oligactis - cnidaria*)	75 mg/l

3.2.1.3.2 Chronic toxicity

Scheubel (1984) reported a 21-day NOEC for lethality and reproduction in *Daphnia magna* of 2.3 mg/l. The first effect on growth was noted at 8 mg/l. The test was a sealed, static test using parallel controls and five test concentrations (0.08-8 mg/l) at 20.5-21.2°C; the results are based on nominal concentrations. The report stated that issues relating to the health and feeding of the *Daphnia* in this type of test could arise which could result in poor test conditions; it was not clear if these occurred with trichloroethylene. It was also not clear from the report whether the solutions were renewed during the test. The study was conducted as a test of the methodology for this type of substance rather than to determine definitive effect concentrations. This test will not be used in the PNEC derivation.

Kordel et al. (1984) reported a 21-day NOEC of 0.15 mg/l for reproduction and mortality in *Daphnia magna* based on nominal concentrations. Inhibition of reproduction was observed at a trichloroethylene concentration of 0.5 mg/l. However, as stated in the report, the test did not fulfil the required quality criteria. Specifically: the concentration of trichloroethylene in solution fell below 80% of nominal within 48 hours; and there were problems with infection of the *Daphnia*. Therefore, this result will not be used in the assessment. (The study was part of a ring test, which concluded that the protocol as used was not suitable for volatile chemicals.)

3.2.1.4 Toxicity to fish

3.2.1.4.1 Acute toxicity

The results of tests on the acute toxicity of trichloroethylene to various fish species are summarised in **Table 3.43**.

Table 3.43 Results of acute toxicity tests of trichloroethylene on fish

Species	Conditions	LC_{50}	Reference
Zebra fish *Brachydanio rerio*	Flow-through, 48 hours	60 mg/l	Slooff (1979)
	Static, 48 hour, covered container.	LC_0 90 mg/l LC_{50} 120 mg/l LC_{100} 150 mg/l	Korte and Greim (1981)
American flagfish Jordanella floridae	Flow-through, 96 hour, 25°C, pH 6.95, hardness 48 mg/l	28.28 mg/l (63.1 mg/l for static test)	Smith et al. (1991)
Dab *Limanda limanda*	Flow-through, 96 hour, 15-20 cm specimens, measured concentration	16 mg/l	Pearson and McConnell, (1975)
Sheepshead minnow *Cyprinodon variegatus*	Static, 96 hour, 22°C, 1.4 mg specimens, measured concentration	52 mg/l	Ward et al. (1986)
Bluegill Lepomis macrochirus	Static, 96 hour, 20-24°C, pH 6.5-7.9, 0.3-1.2 g specimens, nominal concentration, hardness 32-48 mg/l $CaCO_3$	45 mg/l	Buccafusco et al. (1981)
Golden orfe *Leuciscus idus melanotus*	Static, 48 hour, 20°C, pH 7-8, hardness 110 mg/l $CaCO_3$ nominal concentration	136, 203 mg/l	Juhnke and Lüdemann (1978)
Rainbow trout Oncorhynchus mykiss	Static, 48 hour, 15°C, pH 7-8, nominal concentration, hardness 40 mg/l $CaCO_3$	42 mg/l	Slooff et al. (1983)
Killifish *Oryzias latipes*	Static, 48 hour, 20°C, size; 3 cm, 0.3 g specimens, hardness 80 mg/l $CaCO_3$	79 mg/l	Yoshioka et al. (1986)
	Static, 48 hour, 24°C, 4-5 week old fish, Dutch Standard Water, nominal concentration	270 mg/l	Slooff et al. (1983)
Fathead minnow Pimephales promelas	Flow-through, 96 hour, 12°C, pH 7.8-8.0, 1.04 g specimens, measured concentration	40.7 mg/l (static result 66.8 mg/l) EC_{50} loss of equilibrium = 21.9 mg/l	Alexander et al. (1978)
	Flow-through, 96 hour, 30 day old fish, 25 ± 1°C, pH 7.5, measured conc.	44 mg/l	Veith et al., 1983
	Static, 48 hour, 3-4 week old specimens, 20°C, Dutch standard water, nominal concentration	47 mg/l	Slooff et al., 1983
Guppy *Poecilia reticulata*	Semi-static, daily renewal; 7 day exposure; 2-3 month old fish; 22± 1°C	54.8	Könemann (1981)

3.2.1.4.2 Chronic toxicity

Smith et al. (1991) carried out chronic toxicity tests on the American flagfish *Jordanella floridae*. Separate tests were carried out on the embryo/larval lifestage of the fish (fertilised eggs less than 24 hours old) and on week-old fry. Flow-through tests using five duplicate,

logarithmically distributed trichloroethylene concentrations were carried out. The trichloroethylene concentrations were determined throughout the test and the water temperature was maintained at 25 ± 1°C. The embryo/larval stage fish were monitored for hatching success and survival over ten days; the week old fry were assessed on survival and growth over 28 days. Maximum acceptable toxicant concentrations (MATC) were calculated (MATC is the geometric mean of the NOEC and LOEC) for each endpoint:

Egg hatchability	MATC > 21.2 mg/l
10-day larval survival	MATC = 11.0 mg/l
28-day fry survival	MATC = 14.85 mg/l
28-day fry growth	MATC > 20.9 mg/l

The most sensitive of the endpoints studied was larval survival over ten days, which resulted in a NOEC of 5.76 ± 0.77 mg/l.

Loekle et al. (1983) exposed adult black mollies, *Poecilia sphenops*, to concentrations of 0.005 ml/l (7.3 mg/l) or 0.001ml/l (1.5 mg/l) trichloroethylene for 60 days. The solutions in the exposure tanks were changed every two weeks but during this time the trichloroethylene concentration would have decreased due to volatilisation. Severely distressed or dead fish were removed daily. After 60 days, five of the six fish exposed to both doses of trichloroethylene had been removed compared with no removals from the control group. Growth (indicated by total fish weight) was reduced among animals exposed to both concentrations of trichloroethylene, and there were changes in liver morphology of exposed fish. Effects were similar at both concentrations, hence there was no obvious dose-response relationship. A NOEC cannot be derived from this study.

3.2.1.5 Field studies

Lay et al. (1984) carried out a field experiment in a small pond, rich in *Daphnia* and phytoplankton. The pond was compartmentalised using PVC tubing. Four compartments were dosed with trichloroethylene, two with an initial concentration of 25 mg/l and two with an initial concentration of 110 mg/l, while two compartments were used as controls. The trichloroethylene concentration in the dosed compartments decreased rapidly with an estimated half-life of 2.7 days, so that less than 1% of the initial concentration of 110 mg/l remaining after 20 days. Both initial doses of trichloroethylene were toxic to *Daphnia magna*. In the high-dose treatments *Daphnia* were completely removed after 24 and 72 hours, with some recovery towards the end of the experiment (day 40). The low dose had no initial effect (two days), but the population of *Daphnia* decreased over the first four weeks of exposure. Six phytoplankton species were identified before application; three of these showed reduced numbers or elimination, but the numbers of individual of the other species increased.

Lay and Herrmann (1991) investigated the effects of trichloroethylene on plankton in a pond, using transparent enclosures to subdivide the area into four compartments and prevent water exchange with the surrounding pond water. Trichloroethylene was dosed into the enclosure water by diffusion through dialysis membranes for eleven weeks and residues of trichloroethylene and trichloroacetic acid were measured. A “high” concentration of 5-7.5 mg/l was used and a “low” concentration of 1-2 mg/l (the reference states that the trichloroethylene concentration was between 1 and 2 mg/l from the second week of the experiment until the end of the application period but no information is available on the levels immediately after the start of the exposures). The “high” trichloroethylene concentration had effects upon the *Cryptophyceae*, which appeared to be the most sensitive taxa of all algae identified. Although the total phytoplankton density

increased on some days of the exposure period, the primary productivity per cell was significantly reduced at the "high" concentration compared to untreated enclosures. This treatment generally caused a reduction of the population density and of the reproduction of *Daphniae* and *Phyllopodae*. The populations of the *Rotatoriae* species were not generally affected in either treatment. These results suggest a NOEC of 1 mg/l for the test system. However, the results are very difficult to interpret and there is a large variation in controls. The endpoints used are also difficult to incorporate into the standard TGD assessment factor approach. The study will not be used to derive a PNEC value, but will be used as supporting evidence.

3.2.1.6 QSAR values

Hermens et al. (1985b) calculated a 16-day NOEC for *Daphnia magna* according to the Dutch standard method (Concept NEN 6502) of 9.8 mg/l. DeWolf et al. (1988) have calculated a 16-day EC_{50} value for reproduction of *Daphnia magna* of 20.8 mg/l. The calculation was based on the same Dutch standard method.

The ECOSAR computer program has been used to estimate values for ecotoxicity endpoints for various species and these are given in **Table 3.44**.

Table 3.44 Results of ECOSAR

Organism	Duration	Endpoint	Predicted mg/l (ppm)
Green algae	96 hour	EC_{50}	28.9
Green algae	> 96 hour	ChV	3.64
Daphnid	48 hour	LC_{50}	45.2
Daphnid	16 day	EC_{50}	2.76
Mysid	96 hour	LC_{50}	8.89
Fish (Freshwater)	96 hour	LC_{50}	41.0
Fish (Saltwater)	96 hour	LC_{50}	11.4
Fish	14 day	LC_{50}	79.2
Fish	> 14 days	ChV	5.64
Earthworm	14 day	LC_{50}	609

The predicted short-term toxicity values for aquatic organisms suggest a similar sensitivity across the three types of organism (fish, invertebrates and algae).

3.2.1.7 Toxicity to amphibians

Slooff and Baerselman (1980) exposed Mexican axolotl (*Ambystoma mexicanum*) and Clawed toads (*Xenopus laevis*) to various concentrations of trichloroethylene for 2 days at 19-21°C and found LC_{50} values of 48 mg/l and 45 mg/l respectively.

3.2.1.8 Summary of aquatic toxicity

The ecotoxicity test results which will be used to derive the PNEC for the aquatic compartment are summarised in **Table 3.45**.

Table 3.45 Summary of ecotoxicity tests

Test	Reference	Result	Comments
Toxicity to microorganisms	Blum and Speece (1991b)	Methanogens IC_{50} = 13 mg/l	Valid
Acute toxicity to algae	Brack and Rottler (1994)	*Chlamydomonas reinhardii* EC_{50} = 36.5 mg/l	Valid
Acute toxicity to invertebrates	Ward et al. (1986)	*Mysidopsis bahia* EC_{50} = 14 mg/l	Use with care
	Hermens et al. (1984)	*Daphnia magna* IC_{50} = 21 mg/l	Valid
Acute toxicity to fish	Pearson and McConnell (1975)	*Limanda limanda* LC_{50}= 16 mg/l	Use with care
	Veith et al. (1983)	*Pimephales promelas* LC_{50}= 44 mg/l	Valid
	Alexander et al. (1978)	*Pimephales promelas* LC_{50}=40.7 mg/l	Valid
	Smith et al. (1991)	*Jordanella floridae* LC_{50}= 28.3 mg/l	Valid
Chronic toxicity to algae	Brack and Rottler (1994)	*Chlamydomonas reinhardii* EC_{10} = 12.3 mg/l	Valid
Chronic toxicity to invertebrates	Scheubel (1984)	*Daphnia magna* NOEC = 2.3 mg/l	Use with care, not used in PNEC derivation
Chronic toxicity to fish	Smith et al. (1991)	*Jordanella floridae* NOEC = 5.76 mg/l	Valid
Ecosystem test	Lay and Herrmann (1991)	*Daphnia pulex* NOEC = 1 mg/l	Use with care, large variation and unusual endpoints, not used in PNEC derivation

3.2.1.9 Calculation of PNEC for water

There is a significant amount of acute toxicity data available including $L(E)C_{50}$ values for fish, invertebrates and algae. Valid chronic toxicity data are available for fish and for algae. Two chronic toxicity tests are available for *Daphnia magna*, but as discussed in Section 3.2.1.5 neither of these is considered to be valid for use in the risk assessment. There is also an ecosystem study indicating a NOEC of 1 mg/l but this value has not been not used in the assessment due to the variability between the results observed and the unusual endpoints used.

The chronic toxicity test results for fish (NOEC of 5.76 mg/l) and algae (NOEC of 12.3 mg/l) are used to derive the PNEC. A factor of 50 can be used with two long-term NOECs if they include the most sensitive species from the acute studies. Although the lowest actual acute toxicity result is for an invertebrate species, the overall acute results for fish and invertebrates are very similar. This is also seen in the QSAR predictions. It is therefore considered that a factor of 50 can be applied to the long-term fish result in this case. This gives a $PNEC_{water}$ of 115 μg/l.

3.2.1.10 Calculation of PNEC for microorganisms in wastewater treatment plants

Chemicals can have an adverse effect on microbial activity in WWTPs. Therefore, a $PNEC_{microorganisms}$ is derived. Short-term measurements equivalent to the retention time of the chemical in the WWTP are preferable.

The assessment factor to be used depends upon the microbial effect data available. If the test has been performed on nitrifying bacteria, the effect concentration may be compared directly with the effluent concentration. For other tests assessment factors in the range of 10 to 100 may be applied.

For trichloroethylene, toxic effects upon microorganisms are observed at threshold levels between 0.81 mg/l (for the nitrifying bacteria *Nitrosomonas* sp.) and 1,200 mg/l. However, the validity of the result obtained with *Nitrosomonas* sp. is debatable. The report states that the accuracy of the test result was questionable and the value was not used further in the authors' analysis. The lowest valid test result is an IC_{50} of 13 mg/l for bacteria (Blum and Speece, 1991b). Applying an assessment factor of 10 to this result gives a $PNEC_{microorganisms}$ of 1.3 mg/l.

3.2.1.11 Calculation of PNEC for sediment-dwelling organisms

In the absence of measured data on the toxic effects of trichloroethylene on sediment dwelling organisms the equilibrium partitioning method is used to calculate a $PNEC_{sed}$. In using this approach the following assumptions are made:

- Sediment dwelling and aquatic organisms are equally sensitive to the chemical.
- The concentration in sediment, interstitial water and benthic organisms are at a thermodynamic equilibrium. The concentration in any of these three phases can be predicted using the appropriate partition coefficient.
- The sediment/water partition coefficient can either be measured or derived on the basis of a generic partition method from separately measured characteristics of the sediment and the properties of the chemical.

The $PNEC_{sed}$ is given by the following equation:

$$PNEC_{sed} = \frac{K_{susp\text{-}water} \cdot PNEC_{water}}{RHOsusp}$$

For trichloroethylene, the $K_{susp\text{-}water}$ is 3.16 and the $PNEC_{water}$ is 115 μg/l. This gives a $PNEC_{sed}$ of 316 μg/kg.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity to terrestrial organisms

The soil-dwelling earthworm *Eisenia foetida* has been exposed to trichloroethylene in a number of studies. Exposure in artificial soil for 28 days at 21±2°C according to OECD guideline 207 resulted in an LC_{50} of >1,000 mg/kg soil (dry weight) (Viswanathan and Korte, 1984). Scheubel (1984) also found that trichloroethylene concentrations up to 1,000 mg/kg soil had no effect on weight increase, lethality or cocoon production over 28 days. Korte and Freitag (1984) also

determined the LC_{50} to be > 1,000 mg/kg soil. Exposure to trichloroethylene on filter paper for 48 hours in the dark at 20°C (Neuhauser et al., 1985) resulted in an LC_{50} of 0.105 mg/cm^2 filter paper.

Inamori et al. (1989) studied the effect of trichloroethylene on the survival and growth of the soil organisms *Aeolosoma hemprichi* (Oligochaeta), *Philodina erythropthalma* (Rotatoria) and *Colpoda* sp. (Protozoa). These organisms were subcultured in medium containing suspended activated sludge; tests were carried out by incubation in the dark at 20°C after the addition of trichloroethylene. Population numbers were determined daily for *Colpoda* and every four days for *A. hemprichi* and *P. erythrophthalma*, and the EC_{50} values were calculated from the specific growth rate. The EC_{50} values were 75 mg/l for *Colpoda* sp., 92 mg/l for *P. erythrophthalma* and 47 mg/l for *A. hemprichi*. It is not clear whether the concentrations measured are for porewater or for sludge.

Turnip (*Brassica rapa*) and oat (*Avena sativa*) plants were exposed to trichloroethylene concentrations up to 1,000 mg/kg soil with no observed effect on growth (Scheubel, 1984). Korte and Freitag (1984) exposed the same species to trichloroethylene and found the EC_{50} to be > 1,000 mg/kg soil.

Dietz and Schnoor (2001) exposed cuttings of hybrid poplar (*Populus deltoides x nigra* DN34) to trichloroethylene in hydroponic solutions. Exposures were carried out in closed vessels in order to reduce volatilisation and maintain concentrations. Solutions were replaced every two days, and the concentrations were confirmed by analysis. The mass of the cuttings was determined after two weeks exposure. The use of water by the plants was also monitored at two-day intervals as a measure of the transpiration rate. The results were presented as the concentration which resulted in no increase in the mass of the plants over the two-week period (118 mg/l) and as the concentration producing a 50% reduction in the transpiration rate over the same period (131 mg/l).

Effects of trichloroethylene on plants via exposure through the air are discussed in Section 3.2.3.1.

3.2.2.2 Calculation of PNEC

The available results of toxicity testing for terrestrial organisms are limited in scope. Only short-term tests have been carried out on earthworms and plants; the lowest LC_{50} value was > 1,000 mg trichloroethylene/kg soil. If an assessment factor of 1,000 is applied to this, the PNEC for the terrestrial compartment is 1 mg/kg.

Although the exposure of plants in hydroponic solutions is not a soil exposure, it could be considered as equivalent to exposure through the pore water. This study also took precautions to reduce the possible volatilisation from the exposures. The two-week exposures could be considered as acute tests; applying a factor of 1,000 to the lower result of 118 mg/l gives a value of 118 μg/l. This is very similar to the PNEC for aquatic organisms; converting this to a concentration in soil would give the same result as the equilibrium partition method on the aquatic PNEC as calculated below.

The PNEC for the terrestrial compartment has also been calculated using the equilibrium partitioning method in the TGD document using the following equation:

$$PNEC_{soil} = \frac{K_{soil\text{-}water} \cdot PNEC_{water} \cdot 1{,}000}{RHO_{soil}}$$

where:
$PNEC_{water}$ = PNEC for water based on ecotoxicological data for aquatic organisms (= 115 μg/l)
$K_{soil\text{-}water}$ = soil-water partition coefficient = 2.99
RHO_{soil} = density of wet soil, 1,700 kg/m^3

Therefore, the $PNEC_{soil}$ is 202 μg/kg. As the results from tests on terrestrial organisms do not allow a PNEC to be fully defined, the equilibrium partitioning value will be used in the assessment.

3.2.3 Atmosphere

3.2.3.1 Biotic effects through atmospheric exposure

Possible effects of trichloroethylene on plants, especially conifers, have been investigated. Frank and Frank (1985) exposed a 10-year-old spruce tree to trichloroethylene and tetrachloroethylene in the field (Black Forest). Exposures were uncontrolled, the substances being allowed to evaporate from a bottle below the tree, but air samples were taken from between the branches of the tree at 1 m above the source at intervals for analysis. The effect observed was bleaching of chlorophyll from sun-exposed surfaces. This only occurred in needles on the upper face of twigs and only during sunny periods. There was some recovery of partly damaged needles during cloudy periods. Needles on shaded twigs remained dark green. Similar symptoms were seen on the sun-exposed leaves of a hornbeam shrub at a distance of 2 m from the pine tree. The authors suggest that the combined action of the chloroethylenes and UV light was required. As UV is attenuated at lower altitudes by smog, etc., the effect is only observed at higher altitudes. They comment that effects due to other halogenated species or synergism with other pollutants such as sulphur dioxide or nitrogen oxides could not be ruled out; these other species were not monitored.

Frank and Frank (1986) investigated further the possible link between the destruction of photosynthetic pigments and exposure to chloroethylenes, by exposing single needles from spruce trees to airborne concentrations of trichloroethylene under direct irradiation. They found that the needles changed colour from dark green to a dirty brown green (in a 5-hour exposure). The concentrations of pigments in the exposed needles were found to be reduced, particularly chlorophyll-a and β-carotene. Exposure to the higher concentration did not have any greater effect than the lower concentration. The nature of the radiation used in this study has been questioned, as it may have contributed significant amounts of radiation sufficiently energetic to cause direct photolysis of the substance. The report also observes that exposure to UV alone led to a reduction in one of the pigments studied, so it is possible that the needles were under stress as a result of the UV exposure alone.

These studies appear to indicate a possible effect of trichloroethylene on plant pigments under certain circumstances. However it is not possible to determine any meaningful exposure concentrations or conditions from these studies. The same conclusion applies to

tetrachloroethylene. As part of the risk assessment on tetrachloroethylene, a large-scale study was carried out exposing a variety of plants to a range of concentrations of tetrachloroethylene. It was agreed that the results from this study would also be used in the assessment of trichloroethylene. A description of the study is included in the tetrachloroethylene risk assessment report, and full details are contained in Plant Research International (2000). Only a brief summary is included here.

Twelve plant species were selected for the study, including trees, crops, grasses and mosses. Exposures took place for the lifetime of the plants or their growing season, whichever was longer, in 1999. For some species, including trees and mosses, the plants were monitored over the following winter and through to the next spring to look for delayed effects. Five exposure levels were used, as well as a control, and exposures took place in open-topped chambers. Concentrations of tetrachloroethylene in air were monitored regularly. A range of observations and measurements were made on the plants during the exposures, and afterwards for some species. For two of the species (beans and clover) second exposures were carried out in the late summer to follow those in the spring.

The PNEC derived for tetrachloroethylene from this study was 8.2 $\mu g/m^3$. In order to read across to trichloroethylene, this is expressed on a molar basis, as $4.9 \cdot 10^{-8}$ moles/m^3. Assuming a similar potency for trichloroethylene gives a PNEC for plants of 6.5 $\mu g/m^3$.

Both tri- and tetrachloroethylene are chlorinated solvents, so that if the effects on plants are due to their solvent properties this extrapolation seems reasonable. Dietz and Schnoor (2001) exposed cuttings of hybrid poplar (*Populus deltoides x nigra* DN34) to a range of chlorinated solvents, including tri- and tetrachloroethylene, in hydroponic solutions. The results indicated that trichloroethylene may not be as toxic as tetrachloroethylene by this route, the effective concentrations being different by around a factor of three. However, it is not clear whether the route of exposure could influence the toxicity. It is also possible that the effect of tetrachloroethylene on plants is due to the breakdown product trichloroacetic acid (TCA). The corresponding acid from trichloroethylene, dichloroacetic acid, appears to have similar toxicity to TCA, but there may be differences in the extent to which it may be formed in plants. It might therefore be possible to revise the PNEC for trichloroethylene by performing an extensive plant toxicity study. However, it is very uncertain whether a significantly different PNEC would be obtained, and so this is not considered worthwhile.

3.2.3.2 Abiotic effects

Trichloroethylene absorbs infrared radiation at wavelengths which are able to pass through the earth's atmosphere (800-1200 cm^{-1}). However, because of its relatively short half-life in the atmosphere and the low environmental concentrations typically found, its contribution to global warming can be considered to be minor. Trichloroethylene in the atmosphere is unlikely to reach the stratosphere and so is not likely to have an effect on stratospheric ozone. The Photochemical Ozone Creation Potential (POCP) for trichloroethylene is 6 (Derwent and Jenkins, 1990) which is very low (ethylene POCP = 100). Therefore, trichloroethylene is not expected to contribute significantly to photochemical ozone formation.

Snelson et al. (1978) estimated that the breakdown product of trichloroethylene, dichloroacetyl chloride, would have a half-life of > 20 years in the troposphere and potentially could persist long enough to enter the stratosphere. However, this estimate considers only photodegradation. Dichloroacetyl chloride is readily hydrolysed to dichloroacetic acid, which is removed from the

atmosphere in rain. Hence there should be no effect on stratospheric ozone from this breakdown product.

3.2.4 Secondary poisoning

Trichloroethylene is not likely to bioaccumulate (see Section 3.1.2.4), but is not biodegradable (see Section 3.1.2.1.2), and has the proposed classification R45 and R68. Therefore a risk assessment for secondary poisoning is required. There are no reports of dietary toxicity studies with birds, and so an effect concentration has to be derived from the results of studies on laboratory mammals. From Section 4 of this assessment, the no-observed adverse effect level (NOAEL) for chronic exposures is 50 mg/kg day for rats and mice; this is for effects on the kidney. The NOAEL for developmental effects is 350 mg/kg/day for mice and 75 mg/kg/day for rats. The NOAEL for secondary poisoning is therefore taken as 50 mg/kg/day.

From the TGD, the conversion factor from mg/kg/day to mg/kg in food is 20 for the rat and 8.3 for the mouse. These give NOEC values of 1,000 mg/kg for rats and 415 mg/kg for mice. The lower of these values is taken as the NOEC for secondary poisoning. The experiment from which this result came was a 52-week exposure, so an assessment factor of 10 is applied to the result. The PNEC for secondary poisoning is therefore 42 mg/kg in food.

3.3 RISK CHARACTERISATION

As noted in Section 2, the quantities considered to be produced and used in the EU for this assessment are based on data from 1993-1996. It is recognised that some changes have occurred to the overall tonnages and the relative amounts in each area since this time. This needs to be kept in mind in interpreting the conclusions. These changes are most likely to affect the regional concentrations. However, the local concentration estimates are based on more specific information relating to emissions and/or to the sizes of sites using trichloroethylene, and these are considered to be less likely to have changed significantly.

3.3.1 Aquatic compartment (including sediment)

3.3.1.1 Water

PEC/PNEC ratios for the aquatic compartment can be calculated for the local scenarios. The PNEC was calculated to be 115 μg/l. The various $PEClocal_{water}$ values calculated earlier are given in **Table 3.46** along with the PEC/PNEC ratios.

Table 3.46 Local PEC/PNEC ratios for water

Source	$PEClocal_{water}$ (μg/l)	PEC/PNEC
Site-specific (code)		
1A	19.4	0.17
1B	0.4	0.003
1C	0.45	0.004
3	0.38	0.003
4	0.65	0.006
5A	66	0.57
5B	0.4	0.003
Use as intermediate	28.4	0.25
Handling (formulation as a solvent)	1.4	0.012
Metal degreasing	19.6	0.17
Adhesives: formulation	4.5	0.04
Adhesives: use	0.35	0.003
Consumer products: formulation	2.3	0.02
Consumer products: use	0.62	0.005
Others	0.48	0.004

The PEC/PNEC ratio can also be calculated for surface water in the regional and continental compartments:

$PEC/PNECregional_{water} = 3.2 \cdot 10^{-3}$
$PEC/PNECcontinental_{water} = 3.5 \cdot 10^{-4}$

All the ratios calculated are less than 1. Therefore, trichloroethylene is not expected to produce adverse effects in the aquatic (surface water) environment considering releases at all stages of its production and use.

In Section 3.1.3.3 a value of 10 μg/l was selected as representative of the higher end of measured values, the vast majority of measurements being below this value. The environmental quality standard for trichloroethylene is also 10 μg/l. This concentration gives a PEC/PNEC ratio of 0.09. Thus measured levels also indicate that there should be no adverse effects.

Result

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

3.3.1.1.1 Risk characterisation for dichloroacetic acid

In Appendix B the potential risk to the terrestrial compartment from dichloroacetic acid (DCA) formed from trichloroethylene is assessed. The aerial deposition of dichloroacetic acid would be expected to contribute a disperse release to surface water. There are no measurements of concentrations in surface waters. The $PNEC_{water}$ estimated is 0.72 μg/l. All the values measured in rainfall in south western Germany were below this value, as were the mean values measured in Finland. Levels higher than this have been measured in Zurich, but do not take any dilution into account. The levels of dichloroacetic acid appear to decrease rapidly away from assumed anthropogenic sources of trichloroethylene, reaching levels close to the background within mainland Europe. It therefore appears that dichloroacetic acid derived from trichloroethylene will not have any widespread impact on the aquatic environment. It is possible that higher levels could be generated close to sites of emission of trichloroethylene. These would be diluted in surface water, and dichloroacetic acid is expected to degrade; therefore it seems unlikely that significant levels of dichloroacetic acid would persist from this source. However there are no surface water measurements to confirm this (any measured surface water levels would need to be considered carefully as there is clearly the potential for dichloroacetic acid production from chlorination of water).

Result

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

3.3.1.2 Risk characterisation for WWTP

For microorganisms in wastewater treatment plants a $PNEC_{microorganisms}$ of 1.3 mg/l has been calculated. The highest PEC for a wastewater treatment plant calculated in Section 3.1.3.1.1 was 660 μg/l. These give a PEC/PNEC ratio of 0.51. Therefore effects on microorganisms in WWTPs are not expected.

Result

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

3.3.1.3 Sediment

The PNEC calculated for sediment is 316 μg/kg. The highest PEC calculated for sediment from a production site for trichloroethylene is 181 μg/kg. The PEC/PNEC ratio is 0.57.

The PEC/PNEC ratios for the regional and continental compartment are:

$PEC/PNECregional_{sediment} = 3.3 \cdot 10^{-3}$
$PEC/PNECcontinental_{sediment} = 3.6 \cdot 10^{-4}$

These results suggest that trichloroethylene is unlikely to cause any adverse effects in sediment.

Result

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

3.3.2 Terrestrial compartment

The PNEC for the terrestrial compartment has been calculated by the equilibrium partition method as 202 μg/kg. The highest PEC calculated (based on a production site combining the highest actual releases to water and to air) was 31 μg/kg. The ratio PEC/PNEC is 0.15, which suggests that adverse effects on the terrestrial compartment are not likely to occur.

Groundwater levels in contaminated areas can be much higher than those in surface waters. The risk to groundwater has not been addressed in this assessment.

Result

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

3.3.2.1 Risk characterisation for dichloroacetic acid

In Appendix B, the potential risk to the terrestrial compartment from dichloroacetic acid (DCA) formed from trichloroethylene is assessed. There are some recent measured levels for DCA in soil; the range of average concentrations was 0.16-0.54 μg/kg, and the overall average was 0.23 μg/kg. The highest measured value was 0.85 μg/kg. The concentration in soil from deposition of dichloroacetic acid has also been estimated as 0.06 μg/kg, or 0.024 μg/kg if leaching is included. There are no test results for soil organisms, but a PNEC for soil has been estimated from the value for trichloroacetic acid as 1.9 μg/kg. These data would indicate no concern.

Result

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

3.3.3 Atmosphere

Trichloroethylene is thought to make only a minor contribution to global warming. It is likely to have little effect on stratospheric ozone and will not make a significant contribution to photochemical ozone formation.

Section 3.2.3.1 presented studies on the effects of trichloroethylene on plants, from which a PNEC of 6.5 μg/m^3 was calculated by analogy with tetrachloroethylene. Concentrations in air were calculated in Section 3.1.5. The resulting PEC/PNEC ratios are in **Table 3.47**.

Table 3.47 PEC/PNEC ratios for plants exposed through the air

Process	PEClocal$_{air}$ (μg/m^3)	PEC/PNEC
Production - largest site specific	65	10
Use as intermediate	16	2.5
Handling (formulation as a solvent)	92	14
Metal degreasing	10	1.5
Adhesives: formulation	2.4	0.37
Adhesives: use	5.8	0.89
Consumer products: formulation	1.4	0.22
Consumer products: use	0.63	0.1
Others	0.55	0.08
Regional	0.47	0.07

Result

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

This conclusion applies to adhesives formulation and use, consumer product formulation and use, and use in other areas. (It also applies to some specific production and intermediate use sites.)

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

This conclusion applies to the risk of harm to plants from air emissions of trichloroethylene from sites producing trichloroethylene, from sites using trichloroethylene as an intermediate, from sites formulating trichloroethylene as a solvent ("handling"), and from use in metal degreasing. The conclusion does not apply to all production sites or to all sites using trichloroethylene as an intermediate; risks are indicated for two production sites based on measured levels or emissions, and for intermediate sites where specific emission information is not available.

The PNEC on which this conclusion is based is derived from that for tetrachloroethylene on the basis that both substances would be expected to have similar effects.

It should be noted that the conclusions of the human health risk assessment will require risk reduction action to be taken for this substance. The Solvent Emissions Directive (1999/13/EC) will also have an impact on the emissions of this substance.

A risk characterisation for dichloroacetic acid formed as a result of the breakdown of trichloroethylene in the atmosphere is included in Appendix B (related to the terrestrial compartment). The conclusions are given earlier in this section.

3.3.4 Secondary poisoning

PEC values for secondary poisoning were calculated in Section 3.1.6, and the highest concentration was 513 μg/kg in fish. The PNEC for secondary poisoning calculated in Section 3.2.4 was 42 mg/kg in food. The PEC/PNEC ratio is 0.012, and therefore there is no risk from secondary poisoning for any of the steps in the life cycle of trichloroethylene.

Result

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

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Humans may be exposed to trichloroethylene occupationally, via consumer products or indirectly via the environment. Occupational exposure may occur during manufacture, recycling or in the user industries - metal cleaning, adhesives, and use as a chemical intermediate. Occupational exposure may occur via the inhalation and dermal routes. Potential abuse, for example ingestion of trichloroethylene, has not been considered.

Although a substantial amount of trichloroethylene is sold for consumer use in the European Union, the only use identified is for spot cleaning of fabrics. Consumer exposure may occur via the inhalation and dermal routes during or following use as a spot cleaner. Again, potential abuse has not been considered.

Exposure via the environment has been modelled in Section 3.1.7, concerned with environmental exposure. Exposure has been modelled on a regional and a local scale and takes account of exposure via the air, drinking water and a range of foodstuffs. The regional exposures are derived from environmental distributions calculated by the USES model. The local exposures make use of both calculated local emissions and measured values in certain foodstuffs to calculate a worst reasonable case uptake.

4.1.1.2 Occupational exposure

During the manufacture of trichloroethylene and its use in chemical synthesis, workers may be exposed by the inhalation and dermal routes. Inhalation exposure to the vapour is likely where operators breach the closed plant or as a result of spillages. Dermal exposure may occur where workers come into contact with surfaces contaminated by splashes or condensed vapour or as a result of direct splashes on to the skin.

During the use of trichloroethylene, workers may again be exposed by the inhalation and dermal routes. Inhalation exposure to the vapour will occur during activities such as metal cleaning and the use of adhesives. This exposure is likely to be more significant than during manufacture and use in chemical synthesis (that is, use as an intermediate) as these are carried out in closed plant. Dermal exposure may also occur where workers come into contact with surfaces contaminated by splashes or condensed vapour or as a result of direct splashes on to the skin. This may be particularly evident where operators handle degreased components or directly handle adhesives.

The air sampling data presented in the following sections is, with the exception of results obtained from an Italian manufacturing plant, all from UK companies. Consequently the exposure assessments are based on the experience of UK companies. Professional judgement was used where there were gaps in information and it is assumed in the absence of further knowledge that these exposure assessments are representative of all EU member states.

No dermal exposure data were available from industry, so the Estimation and Assessment of Substance Exposure (EASE) model was used to predict exposure via this route. The EASE model was also used where measured inhalation exposure data were not available and it was appropriate to use the model.

The number of workers exposed to trichloroethylene throughout the EU is estimated to be in excess of 60,000, with about 10,000 of these in the UK.

4.1.1.2.1 Manufacture of trichloroethylene

It is understood that approximately 75 persons (including maintenance operators) are exposed to trichloroethylene during its UK manufacture and transport through the manufacturing plant. The number exposed does not include outside contractors who might be working on the plant, which could be up to 60 workers.

Production is carried out in fully enclosed process plant, with occupational exposure occurring during material sampling, maintenance and from spills and leaks. **Table 4.1** represents the results of routine air sampling provided by the UK producer.

Table 4.1 Personal exposures to trichloroethylene during its manufacture in the UK (8-hr TWAs)

Job	Number of results	Geometric mean (ppm)	Maximum (ppm)
Process operators	584	0.6	49
Maintenance	171	0.5	128
Overall for plant	837	0.6	128

These air samples were taken over a three and a half-year period from January 1991. The overall total of 837 personal samples for the plant includes those for workers such as electricians who are not classified as process operators or maintenance personnel.

70% of all the 8-hour time weighted average (TWA) exposures were less than 1 ppm and 98.5% of results were below 10 ppm. Only 13 results were found to be in excess of 10 ppm, with only one of these results above 100 ppm. This result was for plant maintenance and the operator was supplied with respiratory protective equipment. It is understood that where maintenance requires the breaking into production lines the area is first decontaminated and respiratory protective equipment is supplied where significant exposure is possible.

At the UK manufacturing plant the trichloroethylene is piped to the packing area where it is either metered into road tankers or into 210 litre drums. Occupational exposure during tanker filling is minimal, as the tanker driver is remote from the filling point. Drumming is semi-automatic, with the correct volume automatically metered into the drum after the operator places the feed pipe in it. Extraction ventilation is in place at the point of filling. Approximately 10 workers are exposed to trichloroethylene on the packing plant (excluding tanker drivers). Routine air sampling is carried out by the company during packing. These results are shown in **Table 4.2** and are from samples taken over a three and a half-year period from January 1991.

Table 4.2 Personal exposures to trichloroethylene during packing (8-hr TWAs)

Job	Number of results	Geometric mean (ppm)	Maximum (ppm)
Packing	298	1.3	590

42% of the 8-hour TWA exposures were below 1 ppm and 91% of results were below 10 ppm. Only 4.4% of results were above 50 ppm, with 9 results above 100 ppm. These 9 high exposures were thought by the company to be due to spillages. It was reported that in these situations the operators were equipped with respiratory protective equipment.

Trichloroethylene is manufactured at two plants in Italy, with a total of 70 employees exposed. Manufacture is carried out in closed plant and steps are taken to avoid leaks by routine plant maintenance. Personal protective equipment is worn where necessary. **Table 4.3** shows the results of static sampling carried out by the firm at the two manufacturing plants in 1993. These samples were taken over 2-4 hour periods. Details of the number of samples and locations of the sampling equipment were not provided.

Table 4.3 Static air sampling results for trichloroethylene during manufacture in Italy

Site	Mean (ppm)	Range (ppm)
1	2.7	0.2-9.1
2	0.5	0.1-1.5

The total number of workers exposed during manufacture and packing throughout the EU has not been established. However, assuming similar numbers of workers are exposed at each of the 5 manufacturing plants it is estimated that 400-700 workers (including maintenance personnel and contractors) are exposed during manufacture and packing of trichloroethylene.

A small amount of re-bottling of trichloroethylene is carried out where suppliers are providing small volumes for specific cleaning applications. Air sampling data have not been obtained for this application. However, exposure is likely to be low as it is carried out in closed bottling plants to avoid loss of product.

Exposure data for short-term activities were not available. It is likely that for short duration activities such as unplanned or planned maintenance, where there is the potential for high short-term exposures, involve the use of respiratory protective equipment and probably purging of the plant. Where such controls are in place high short-term exposures, in excess of exposure limits, should not occur.

The value taken forward to the risk characterisation will be 10 ppm 8-hour TWA.

Dermal exposure can occur during manufacture, however this is only likely to occur during activities such as sampling and maintenance, when operators would be expected to wear gloves.

The best EASE scenario for this exposure is direct handling with incidental contact, where incidental refers to one significant contact in a shift, for example spilling trichloroethylene whilst taking samples or touching a wet surface. This results in a prediction of 0 to 0.1 mg/cm^2/day, although on most days no such accidental contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the

bottom of this range. The maximum surface area likely to be exposed will be the area of two hands (820 cm^2).

4.1.1.2.2 Recycling trichloroethylene

Only a few companies are understood to recycle trichloroethylene in the EU. Therefore the numbers exposed during this work are relatively small. Furthermore, as the recycling is carried out in closed vessels, exposure is likely to be low. One UK company provided results of routine long-term static sampling and short-term colorimetric detector tube measurements taken on their recycling plant. The long-term static samples were taken using the method detailed in MDHS 72 (Volatile organic compounds in air - Laboratory method using pumped solid sorbent tubes, thermal desorption and gas chromatography) which yielded results of <1 ppm to 9 ppm (mean, 2.7 ppm). The short-term colorimetric detector tube measurements gave results of <2 ppm to 50 ppm (mean, 12 ppm).

In the absence of further details on the results (i.e. 90 percentiles), 9 ppm 8-hour TWA will be taken forward for the risk characterisation. For short-term exposure a value of 50 ppm 15-min TWA will be used.

Dermal exposure can occur during recycling but like manufacture, this is only likely during activities such as sampling and maintenance, when operators would be expected to wear gloves.

The best EASE scenario for this exposure is direct handling with incidental contact, where incidental refers to one significant contact in a shift, for example spilling trichloroethylene whilst taking samples or touching a wet surface. This results in a prediction of 0 to 0.1 mg/cm^2/day, although on most days no such accidental contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range. The maximum surface area likely to be exposed will be the area of two hands (820 cm^2).

4.1.1.2.3 Metal cleaning

It is estimated that there are about 6,000 hot vapour degreasing units in use in the UK. Each is operated by 1 or 2 workers, resulting in a total of 5,000 to 10,000 exposed workers. However, the nature of the work is such that the degreasing bath could be used by any number of workers during a shift. Therefore the actual number exposed is likely to be significantly higher than the above.

The number of degreasing units in use in the EU is not known. However, the UK is understood to use approximately a sixth of the total trichloroethylene used by member states. It is therefore estimated that there may be 30,000 to 40,000 degreasing units in use in the EU and the number of workers exposed could be in excess of 60,000.

HSE data

The limited amount of data on the HSE National Exposure DataBase (NEDB) is shown in **Table 4.4**. These results are from 12 routine visits carried out by HSE inspectors between 1984 and June 1994.

Table 4.4 Personal exposures during degreasing - HSE data (8-hr TWAs)

No of results	<30 ppm	<50 ppm
25	96%	100%

Industry data

The last major survey of occupational exposure during the operation of degreasing baths, to which many suppliers still refer, was carried out in the late 1970s (Shipman and Whim, 1980). The results of this work are reproduced in **Table 4.5**.

Table 4.5 Personal exposures during degreasing (8-hr TWAs) (Shipman and Whim)

No of results	< 30 ppm	< 50 ppm	< 100 ppm
212	91%	97%	99%

This survey covered 25 locations, with a total of 32 installations. Only one result was reported to be in excess of 100 ppm, which was due to the work piece being removed from the bath too quickly. The report concluded that operator exposure can be very low and that high exposures are due to:

a) incorrect siting of the plant,
b) excessive drag out due to incorrect operation,
c) inadequate plant maintenance,
d) overloading of equipment and incorrect jigging of work leading to solvent trapping.

The results of this work were also published in the Handbook of Occupational Hygiene (Kluwer Publishing Limited, 1983). A further 94 results were included in this report from a further 25 installations at a further 12 locations. The total results for the initial work and these additional measurements are listed in **Table 4.6**.

Table 4.6 Personal exposures during degreasing (8-hr TWAs) (Shipman and Whim including additional data)

No of results	< 30 ppm	< 50 ppm	< 100 ppm
306	86%	94%	96%

The amount of data available from industry other than the above work is relatively small considering the scale of use of trichloroethylene. Where data are available it appears that many companies, even large-scale users, have relied on short-term colorimetric detector tubes for obtaining measurements.

Measurements using short-term colorimetric indicator tubes obtained from one major user of trichloroethylene showed exposure to range from 20 to 50 ppm during loading and unloading of components. Where control was poor, airborne concentrations of up to 500 ppm were found. High exposures (in excess of 100 ppm) were generally as a result of a failure to maintain the controls or as a result of poor working practices.

The results of the survey carried out in the late 1970s and HSE's NEDB suggest that 8-hour TWA exposure can be controlled below 50 ppm. Although based on UK data this work suggests exposure can be controlled below the majority of current EU occupational exposure limits and for a well maintained and well operated degreasing bath exposure can be as low as 20 ppm (8-hour TWA). However, the results of short-term detector tube measurements obtained from industry suggests that in many situations exposures are in excess of the current UK Short Term Exposure Limit (STEL) of 150 ppm which may in turn result in significant shift exposures. Results of short-term detector tube measurements taken by the HSE in 1994, during a programme of 170 visits specifically looking at degreasing baths, have also shown high airborne concentrations on many occasions. These high exposures were where companies had failed to adequately maintain degreasing baths or where working practices were poor. It therefore appears that, although it has been shown that exposure can be controlled to as low as 20 ppm (8-hour TWA), industry is in general failing to meet current standards. The technology to control exposure to this level has been available for many years, though newer baths may have improved controls such as deeper free board zones and improved extraction. Failure to control exposure may be for any one or a combination of the reasons detailed earlier in this review. Occupational exposures are likely to be lower than the above values during the use modern enclosed degreasing baths.

Occupational exposure to trichloroethylene can also occur during the cleaning out of degreasing plants. Air sampling results were obtained from one manufacturer (**Table 4.7**) where a direct reading instrument was used to measure personal exposure during a clean out operation. This exercise was carried out early in 1994. The procedure involves distilling the solvent into a drum with extraction ventilation at the filling point and then draining the residues. Any residual slurry can be scraped out though an access point at the base of the degreasing bath avoiding the need to enter it. This operation takes approximately 45 minutes. The results of the exercise were as follows. These results represent exposure where cleaning is carried out to best practice.

Table 4.7 Personal exposure during cleaning out of a degreasing bath (industry data)

	Mean conc. during test period (ppm)	Highest conc. during test period (ppm)	Lowest conc. during test period (ppm)
Distilling out the trichloroethylene	27	109	6.3
Draining residues with extraction on	59	127	27
Draining residues with extraction off	78	132	25

HSE undertook a succession of air sampling surveys (1994/95) during the cleaning of degreasing baths. These are during cleaning operations where the operator does not enter the degreasing bath. The results of the first of these surveys are detailed in **Table 4.8**.

Table 4.8 Personal exposure during cleaning of a degreasing bath (HSE data)

No of results	Range	Mean	% > 100 ppm	% > 150 ppm	% > 300 ppm
5	9 - 350	160	60	40	20

These air samples were taken over periods of 18 minutes and represent short-term exposure during cleaning. This air sampling was carried out during a typical cleaning exercise and indicates that exposures are not adequately controlled by engineering means when compared against the UK STEL of 150 ppm. Respiratory protective equipment is generally therefore still necessary during this cleaning. Suppliers recommend that cleaning is carried out by the above method with the operator only entering the degreasing bath if this is not practicable. Airborne concentrations in excess of 5,000 ppm have been estimated where operators, not wearing the necessary protective equipment, have been overcome whilst working inside degreasing baths. Improvements are being made by degreasing bath manufacturers to remove the need for operators to enter the bath. Where operators do enter a degreasing bath to clean, adequate control of exposure can only be achieved by the provision of suitable breathing apparatus.

The values of 50 ppm 8-hour TWA and 500 ppm 15-min TWA will be taken forward for the risk characterisation. The former represents exposure over a full working shift that is likely to represent what is being achieved by most plants (i.e. approximates to the 90/95 percentiles). The latter represents short-term exposure during bath loading / unloading that has been found during these activities, and probably represents exposures at many plants.

Dermal contact is likely to be significant during use as a metal cleaner, as operators handle wet components. The degree of this contact will depend on the working practices and precautions taken. Dermal exposure data were not available from industry so the EASE model was used to predict exposure. This predicted dermal exposure of 0.1-1.0 mg/cm^2/day, assuming that the operator comes into contact with the trichloroethylene intermittently. The value of 1.0 mg/cm^2/day has been adopted as a worst reasonable case exposure via the dermal route.

4.1.1.2.4 Adhesives

Manufacture

It is estimated that there may be up to 10 to 15 companies producing trichloroethylene based adhesives in the UK. The total number of manufacturers producing trichloroethylene based adhesives in the EU has not been established. Adhesive manufacture tends to involve small batch processes where 2/3 operators may be exposed. The formulating is generally carried out in closed mixing vessels with exposure during activities such as charging, sampling and discharging.

Data were not available from industry for exposure during manufacture of adhesives. Generally, companies reported that trichloroethylene is used infrequently and not included in routine air monitoring programmes. Air sampling for solvents used more frequently is carried out to demonstrate adequate control of exposure.

Where preparation of the adhesive is carried out in open mixing vessels exposure may be more significant, with the level of exposure dependant on whether LEV is used or not. Where LEV is used the EASE scenario that best describes this is non-dispersive use with LEV, which results in a prediction of 10 to 20 ppm 8-hour TWA. Where LEV is not used the EASE scenario that best describes this is non-dispersive use with direct handling with dilution ventilation, which results in a prediction of 100 to 140 ppm 8-hour TWA. The use of LEV can be considered to be the minimum standard of control for this use, therefore for most plants exposures of up to 140 ppm 8-hour TWA can be considered to be an over estimate. Exposures during charging / discharging where mixing is in closed vessels is likely to be lower than these values. It is also worth noting that the above predictions assume that the operator is only exposed to trichloroethylene vapour

and spends the full shift near the mixing vessel. Since only a proportion of the blend will be trichloroethylene and the operator is unlikely to be near the vessel for the full shift, the above exposures are likely to be overestimates.

Dermal contact may be significant during manufacture of adhesives, as operators may come into contact with it quite frequently. The degree of this contact will depend on the working practices and precautions taken. Dermal exposure data were not available from industry so the EASE model was used to predict exposure. This predicted dermal exposure of 0.1-1.0 mg/cm^2/day, assuming that the operator comes into contact with the trichloroethylene intermittently (the same scenario as metal degreasing). The value of 1.0 mg/cm^2/day has been adopted as a reasonable worst-case exposure via the dermal route.

Use

During use of the adhesive the workers may be exposed to the trichloroethylene vapour. Trichloroethylene is used in adhesives where a solvent of low flammability with the desired drying time is required. Applications for these adhesives are numerous, therefore it was not possible to obtain comprehensive exposure data. Exposure may be to small amounts or to high concentrations during extensive use in confined areas. It is unlikely that companies would routinely air sample for trichloroethylene during such uses, although control regimes may include local exhaust ventilation and respiratory protective equipment to reduce exposure.

Dermal contact is likely to be significant during use as an adhesive, as operators may come into contact with it quite frequently. The degree of this contact will depend on the working practices and precautions taken and therefore it was not possible to obtain reliable exposure data.

4.1.1.2.5 Use as an intermediate

Information was only available for the manufacture of HCFC 133a and HFC 134a. The numbers of workers exposed during the manufacture of HCFC 133a and HFC 134a is about 100 (including process operators, maintenance operators and outside contractors). Occupational exposures are low (**Tables 4.9** and **4.10** respectively), as the process is fully enclosed. The results presented are from air sampling surveys carried out over a three and a half year period starting in January 1991.

Table 4.9 Personal exposures during manufacture of HCFC 133a (8-hr TWAs)

Job	Number of results	Mean (ppm)	Maximum (ppm)
Process operators	162	0.2	11.5
Maintenance	7	0.3	1.5

Table 4.10 Personal exposures during manufacture of HFC 134a (8-hr TWAs)

Job	Number of results	Mean (ppm)	Maximum (ppm)
Process operators	57	0.2	1.6
Maintenance	34	0.2	2.7

Exposure data for short-term activities were not available. It is likely that for short duration activities such as unplanned or planned maintenance, where there is the potential for high short-term exposures, involve the use of respiratory protective equipment and probably purging of the plant. Where such controls are in place high short-term exposures, in excess of exposure limits, should not occur.

The value taken forward to the risk characterisation will be 11.5 ppm 8-hour TWA, in the absence of further information about the spread of the data for the above results.

Dermal exposure can occur during manufacture, however this is only likely to occur during activities such as sampling and maintenance, when operators would be expected to wear gloves.

The best EASE scenario for this exposure is direct handling with incidental contact, where incidental refers to one significant contact in a shift, for example spilling trichloroethylene whilst taking samples or touching a wet surface. This results in a prediction of 0 to 0.1 mg/cm^2/day, although on most days no such accidental contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range. The maximum surface area likely to be exposed will be the area of two hands (820 cm^2).

Dermal exposure can occur during use as an intermediate but like manufacture, this is only likely during activities such as sampling and maintenance, when operators would be expected to wear gloves. EASE predictions suggest dermal exposure is insignificant.

4.1.1.2.6 Summary

It is estimated that between 400 and 700 workers in the EU are exposed to trichloroethylene during manufacture. The main route of exposure is via inhalation. Personal exposure data are available from a UK manufacturing plant, for workers involved in production and packing. The majority of 8-hour time weighted average exposures were below 10 ppm. Very occasionally, personal-sampling atmospheric 8-hour TWAs above 100 ppm and as high as 590 ppm were reported, possibly due to spillages, but respiratory protective equipment was worn when such conditions were anticipated. In two Italian plants, static sampling has indicated exposures to atmospheric concentrations of between 0.1 and 9.1 ppm during manufacture.

A relatively small number of workers are exposed during recycling. The main route of exposure is via inhalation. Data from one UK plant gave 8-hour TWA values of <1 to 9 ppm (mean 2.7 ppm). Again, dermal exposure is expected to be insignificant.

For manufacture and recycling dermal exposure was predicted using EASE to be in the range 0 to 0.1 mg/cm^2/day. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range.

The numbers of workers exposed to trichloroethylene in metal degreasing units in the EU could be in excess of 60,000. Workers are likely to be exposed by both the inhalation and dermal routes.

UK data from several sources indicate that workplace inhalation exposures (8-hour TWA) can be controlled to below 20 ppm for a well maintained and correctly operated degreasing bath. Occupational exposures are likely to be lower during the use modern enclosed degreasing baths. However, exposures are often higher than 20 ppm 8-hour TWA during the use of open top degreasing baths. The 90 / 95 percentile approximates to 50 ppm 8-hour TWA for the data

received. For short-term exposure, the results of measurements taken by HSE and industry indicate that exposures during loading/unloading of the degreasing bath are on many occasions above the current UK short-term occupational exposure limit of 150 ppm and may be as high as 500 ppm. These short-term exposures may result in high shift exposures. These high short-term exposures were found where companies had failed to adequately maintain the degreasing bath or where working practices were poor. During the cleaning of degreasing baths where the operator enters the bath short-term exposures in excess of 5,000 ppm have been estimated, although in these situations control of exposure is achieved by the provision of suitable breathing apparatus. Improvements in bath design are removing the need for operators to enter the baths.

No data on dermal exposure are available from industry. Modelled data (using EASE) indicated that exposure could be as high as 1mg/cm^2/day, assuming regular contact and minimal handling precautions; this value has been adopted as the worst reasonable case exposure via the dermal route.

A relatively small number of workers are exposed to trichloroethylene during the manufacture of trichloroethylene-based adhesives. Exposures for the manufacture of adhesives using open mixing vessels were predicted with EASE, although many plants may use closed systems. Where LEV is used on open mixing vessels, 10 to 20 ppm 8-hour TWA was predicted and where LEV is not used 100 to 140 ppm 8-hour TWA was predicted. The use of LEV can be considered to be the minimum standard of control for this use, therefore for most plants exposures of up to 140 ppm 8-hour TWA can be considered to be an over estimate. Exposures during charging / discharging where mixing is in closed vessels is likely to be lower than these values. It is also worth noting that the above predictions assume that the operator is only exposed to trichloroethylene vapour and spends the full shift near the mixing vessel. Since only a proportion of the blend will be trichloroethylene and the operator is unlikely to be near the vessel for the full shift, the above exposures are likely to be overestimates.

Dermal exposure is also possible. Using the EASE model, exposure as high as 1 mg/cm^2 is predicted; this value has been adopted as the worst reasonable case exposure via the dermal route.

During use of the adhesive the workers may be exposed to the trichloroethylene vapour. Trichloroethylene is used in adhesives where a solvent of low flammability with the desired drying time is required. Applications for these adhesives are numerous, therefore it was not possible to obtain comprehensive exposure data. Exposure may be to small amounts or to high concentrations during extensive use in confined areas. It is unlikely that companies would routinely air sample for trichloroethylene during such uses, although control regimes may include local exhaust ventilation and respiratory protective equipment to reduce exposure.

The only information available on use as an intermediate relates to the manufacture of HCFC 133a and HFC 134a. It is estimated that about 100 workers in the EU are exposed to trichloroethylene during the manufacture of these chemicals, via inhalation. Industry sampling surveys indicate 8-hour TWA exposures up to a maximum of 11.5 ppm, with a mean of about 0.2 ppm. Dermal exposure is expected to be similar to manufacture and recycling.

Table 4.11 Summary of occupational inhalation exposure to trichloroethylene (values taken forward to the risk characterisation)

Exposure scenario	Exposure level	Source
Manufacture and recycling	8-hr TWA - 10 ppm short-term peaks - potential for high peaks - cannot quantify	Industry
Metal degreasing	8-hr TWA - 50 ppm short-term peaks - 500 ppm	HSE / published / industry
Adhesives (manufacture)	8-hr TWA - 10 - 20 ppm (with LEV) and 100 -140 ppm (without LEV)	EASE
Adhesives (use)	Wide use, controls uncertain - not quantifiable	N/a
Manufacture of HCFC 133a and HFC 134a	8-hr TWA - 11.5 ppm	Industry

4.1.1.3 Consumer exposure

In 1993, the production of trichloroethylene within the EU was 115,000 tonnes; 78,000 tonnes were sold within the EU. The main use of trichloroethylene is as an industrial metal-degreaser and about 82% of the trichloroethylene was used for this purpose.

About 9% was used for adhesives and associated cleaners for industrial purposes. It appears that there is no consumer use in the EU for these adhesives. A small amount of trichloroethylene-containing adhesive is produced in Europe for consumer use in Africa but this is declining rapidly.

The extraction of fat from meat using trichloroethylene is no longer permitted in the EU. The only consumer use appears to be for spot cleaning fabrics. That is, as a cleaner for small patches of grease or oil on cloth. This accounts for approximately 6% of sales of trichloroethylene, some 4,600 tonnes per annum. Trichloroethylene is sold in glass bottles in many Italian supermarkets (national chains) 99.9% pure, with no instructions on use. They are placed on the same shelves with other household cleaning products. The same product has also been found in supermarkets in Belgium.

4.1.1.3.1 Exposure to trichloroethylene from cloth cleaning

The maximum size of bottles retailed for this purpose is understood to be 500 ml. There are no measured exposure data available on consumer exposure during this cleaning process. A method of modelling the potential exposure is described below.

The exposure scenario is for a single event in which a small volume of cleaning agent is used. Two routes of exposure are considered, inhalation, following evaporation of the solvent during and after application and dermal exposure as a result of skin contact during application.

Inhalation exposure

The US EPA *SCIES* model was selected. *SCIES* is a computerised model for estimating the consumer exposure to chemical substances. The program scenario used is that for the use of an all-purpose cleaner.

The program assumes that the cleaner is used in a room of 20 m^3 volume with connection through ventilation (0.2 air changes per hour) to a house of 292 m^3. The largest bottle of cleaner

available contains 500 ml and each event uses 10% of this quantity. The cleaning lasts for ten minutes and the person involved continues normal domestic activity for the rest of the day. The breathing rate during use is assumed to be 1.3 m^3/hr and after use 1.1 m^3/hr (both are *SCIES* default settings).

Results

SCIES calculates an evaporation time of 0.026 hours (just over 90 seconds), a peak concentration of 2.4 g/m^3 and a potential user exposure of 1.9 g/day.

Dermal exposure

The US EPA *Dermal* model was chosen to predict dermal exposure for the same use scenario, where the trichloroethylene is used as a general purpose cleaner. It is assumed that no protective gloves are being used and all of the chemical is absorbed from a set thickness of cleaner coating all the surface area of the hands.

The surface area of the skin is assumed to be 795 cm^2 and the film thickness $2.14 \cdot 10^{-3}$ cm (both *Dermal* default settings). The volume of cleaning fluid in contact with hands is therefore $2.14 \cdot 10^{-3} \cdot 795 = 1.7$ cm^3. The density of trichloroethylene is 1.4649.

Results

The potential dose from dermal absorption is 2.5 g/day for a single event.

Summary

The only consumer use appears to be for spot cleaning fabrics. That is, as a cleaner for small patches of grease or oil on cloth. There are no measured data available on consumer exposure for this cleaning process, so the exposure was modelled using a scenario for a single event in which a small volume of cleaning agent is used. Two routes of exposure were considered, inhalation, following evaporation of the solvent during and after application and dermal exposure as a result of skin contact during application. For inhalation exposure, the US EPA *SCIES* model was selected; *SCIES* predicted a potential user exposure of 1.9 g/day for a single event. For dermal exposure, the US EPA *Dermal* model was chosen; *Dermal* predicted a potential dose of 2.5 g/day for a single event.

4.1.1.4 Humans exposed via the environment

Indirect exposures and the resulting intakes via the environment were calculated in Sections 3.1.7.1 and 3.1.7.3 for the regional and local levels respectively. The relevant tables, 3.32 and 3.37 are repeated below for completeness as **Tables 4.12** and **4.13** respectively. The regional exposure levels represent a background level of exposure; local exposures are a worst case, using a PEC_{local} prediction for airborne exposure and literature data for contaminant levels in foodstuffs and drinking water. Total intake at the regional level is calculated to be $1.5 \cdot 10^{-4}$ mg/kg/day, at the local level 0.022 mg/kg/day.

Table 4.12 Regional concentrations in air, water and biota and the calculated human intake

Concentrations in air, water and biota intake	Human consumption or intake rate per day	Human intake via indirect exposure (mg/kg/day) [1,2)]
Air = 0.47 μg/m^3	20 m^3	Air = $1.3 \cdot 10^{-4}$
Drinking water = 0.17 μg/l	0.002 m^3	Drinking water = $5.0 \cdot 10^{-6}$
Fish = 6.1 μg/kg	0.115 kg	Fish = $1.0 \cdot 10^{-5}$
Leaf/Stem crop = 0.004 μg/kg	1.2 kg	Stem of plants = $6 \cdot 10^{-8}$
Root crop = 0.015 μg/kg	0.384 kg	Root of plants = $8 \cdot 10^{-8}$
Meat = $3 \cdot 10^{-4}$ μg/kg	0.301 kg	Meat = $1.4 \cdot 10^{-9}$
Milk = $5 \cdot 10^{-4}$ μg/kg	0.561 kg	Milk = $4 \cdot 10^{-9}$
		Total = $1.5 \cdot 10^{-4}$ mg/kg/day

1) Assuming a 70 kg person
2) Assuming an intake of 100%

Table 4.13 Local concentrations in air, water and biota and the calculated human intake

Concentration in air, water and biota	Daily human consumption or intake	Calculated human intake via indirect exposure (mg/kg/day)[1,2)]
Air = 65 μg/m^3 ($PEClocal_{air}$ measured near a production plant)	20 m^3	0.019[3]
Drinking water = 21μg/l (Ballschmiter et al., 1988 , worst case)[2]	0.002 m^3	$6 \cdot 10^{-4}$
Fish = 479μg/kg (Dickson and Riley 1976, dogfish)	0.115 kg	$7.9 \cdot 10^{-4}$
Leaf/stem crop = 6 μg/kg (Bauer, 1981)	1.2 kg	$1 \cdot 10^{-4}$
Root crop = 6μg/kg (Bauer, 1981)	0.384 kg	$3.3 \cdot 10^{-5}$
Meat = 192 μg/kg (Bauer, 1981)	0.301 kg	$8.3 \cdot 10^{-4}$
Milk/dairy products = 20 μg/kg (Heikes, 1987)	0.561 kg	$1.6 \cdot 10^{-4}$
		Total = 0.022 mg/kg/day

1) Assuming a 70 kg person
2) Assuming an intake of 100%

4.1.1.5 Combined exposure

Given the large differences in exposure and the different exposure scenarios for humans via the three different routes, it was not considered useful to produce a combined exposure assessment.

4.1.2 Effects assessment: hazard identification and dose (concentration) - response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

Many studies in both animal models and humans have been published on the toxicokinetics of trichloroethylene including a number of physiologically based pharmacokinetic models. All of the more important studies have been obtained in their original form and critically assessed; less vital but nevertheless useful additional information to complete the picture has been taken from a recent comprehensive and high quality review (Davidson and Beliles, 1991). In this document only the data obtained using routes of exposure relevant to humans under normal conditions of use (i.e. inhalation, oral and dermal routes) have been considered.

4.1.2.1.1 Studies in animals

Inhalation

Absorption

In animals trichloroethylene is readily absorbed through the lungs. Forssman and Holmquist (1953) exposed groups of 4 rats to 10,786 or 16,270 ppm (59 or 86 mg/l) trichloroethylene for 30-60 minutes. They found that 31-79% of the trichloroethylene inhaled was retained. In mice exposed to around 19 mg (10 µl of 0.135 µCi/µl) ^{14}C-trichloroethylene (specific activity 9.3 mCi/mmol) for 10 minutes, uptake assessed as the difference in chamber radioactivity before and after exposure was found to be 40-54% (Bergman, 1979). In recent studies, blood concentrations of trichloroethylene determined immediately post exposure to unlabelled trichloroethylene were found to increase linearly in relation to dose in rats exposed to up to 3,000 ppm for 8 hours (Arito et al., 1993). In this study, exposure to 6,000 ppm for 4 hours produced blood levels equivalent to those found after 8 hours exposure to 3,000 ppm. Similarly, in mice, linear increases in blood concentrations were observed after 30-minute exposures to up to 7,000 ppm (Villaschi et al., 1991).

Blood trichloroethylene concentrations were determined for groups of 3-8 female Sprague-Dawley rats regularly during exposure to unlabelled trichloroethylene at concentrations of 50 or 100 ppm for 2 hours or 200, 400 or 500 ppm trichloroethylene for 6 hours (Jakobsen et al., 1986). Non-linear regression analysis was then used to extrapolate the data to determine steady state concentrations of trichloroethylene in the blood for each exposure concentration. Mean steady state blood concentrations after 2 hours exposure to 50 or 100 ppm were 0.69 and 2.1 µg/ml respectively. At higher airborne concentrations of 200, 400 or 500 ppm after 6 hours exposure, mean steady state blood levels of 5.0, 18.7 and 27.3 µg/ml respectively were found. This suggests that in rats, steady state levels of trichloroethylene in the blood are proportionately greater for higher airborne concentrations.

In a separate study, total body burdens were quantified for groups of 4 male B6C3F1 mice or Osborne-Mendel rats exposed (whole body) for 6 hours to either 10 or 600 ppm (54 or 3,228 mg/m^3) 14C-trichloroethylene (Stott et al., 1982). At the end of the exposure period animals were placed in metabolism cages for the next 50 hours for collection of faeces, urine and exhaled air. In mice exposed to 10 ppm, a mean body burden of 78.5 mmol-equivalents/kg was found, increasing 40 fold to 3,138 mmol-equivalents/kg in mice exposed to 600 ppm. In rats

exposed to 10 ppm, a mean body burden of 35.8 mmol-equivalents/kg was found, increasing 30-fold to 1,075 mmol-equivalents/kg at 600 ppm. These two studies (Stott et al., 1982; Jakobsen et al., 1986), taken together suggest that although blood levels increase in proportion to increasing dose, uptake into certain compartments within the body may be saturated at the higher dose level so that overall body burdens do not necessarily increase in direct proportion to dose.

Trichloroethylene uptake has also been briefly investigated in pregnant goats and sheep where it was found that trichloroethylene could be detected in both the maternal and foetal circulation within 6 minutes of the start of exposure (Helliwell and Hutton, 1950). Uptake was not quantified in this study.

It is therefore reasonable to conclude that pulmonary uptake of trichloroethylene is rapid and extensive and there is no evidence to suggest that transfer of trichloroethylene across the lungs will differ significantly between experimental animal species.

Distribution

There is good evidence from studies in rats and mice that trichloroethylene and/or its metabolites distribute(s) throughout the body. In the most detailed study, autoradiography was used to study the distribution of radioactivity in NMRI mice exposed for 10 minutes to around 353 mg ^{14}C-trichloroethylene (10 µl of 2.5 µCi/µl trichloroethylene, specific activity 9.3 mCi/mmol, by inhalation) and sacrificed 0, 0.5, 1, 2, 4, 8 and 24 hours later (Bergman, 1979). In this study the distribution of volatile and non-volatile radioactivity was compared by exposing hemisections at both -80^{o}C and following heating of the hemisection to 54^{o}C for 24 hours.

Immediately after exposure, predominantly volatile radioactivity was distributed throughout the body, though levels were particularly high in the brain and body fat. Measurable levels of non-volatile radioactivity were only found in the liver, bronchi and kidneys. Half an hour later no radioactivity was detected in the brain indicating rapid elimination from this tissue. In contrast, volatile radioactivity was still measurable in body fat 4 hours after exposure and traces of radioactivity were still present 8 hours post exposure indicating a much longer retention time in fat. Levels of non-volatile radioactivity increased over the next few hours, peaking four hours after exposure in the kidneys and 8 hours after exposure in the liver. The presence of radioactivity in intestinal contents between half an hour and eight hours post exposure was taken to indicate that some biliary elimination was occurring though this could also represent transfer into the gastrointestinal tract from the blood via the gastrointestinal mucosa. Non-extractable radioactivity was found in the liver, bronchi and kidney suggesting that some tissue binding occurred. No analysis of volatile and non-volatile radioactivity was carried out but it is likely that the volatile radioactivity is mainly trichloroethylene and non-volatile and bound radioactivity represents metabolites of trichloroethylene.

More recently low temperature (-80^{o}C) and conventional autoradiography has been used to study the distribution of radiolabel in pregnant mice exposed to ^{14}C-trichloroethylene for 10 minutes on days 11, 14 and 17 of gestation and killed 0, 1, 4 or 24 hours post exposure (Ghantous et al., 1986). In dams, the temporal and spatial distribution of radioactivity was similar to that reported by Bergman above. Again, non-extractable radioactivity was seen in the liver, bronchi and kidney cortex and also in the nasal mucosa. In foetuses, volatile radioactivity could be detected at the one hour but not the four hour time point and levels were always lower than those found in dams. The highest levels of non-volatile radioactivity in foetuses were found at the four-hour time point. At early stages of gestation (day 11) both volatile and non-volatile radioactivity was evenly distributed throughout the foetus. However, at later gestational stages (days 15 and 18) both volatile and non-volatile radioactivity were found to accumulate more in the liver and blood

and levels of non-volatile radioactivity in the developing brain were much lower than those found in day 11 foetuses. There was also a tendency for non-volatile radioactivity to accumulate more in the skeleton and urinary tract at the later time points. At all stages of gestation volatile and non-volatile radioactivity was found in the ventricular cerebrospinal fluid of foetuses after one hour but not after four hours. It was also noted that non-volatile radioactivity was present in amniotic fluid at all time points including the final (24 hour) sampling time. There was no evidence of binding in foetal tissues or amniotic fluid. Again no identification of the chemical nature of the volatile and non-volatile radioactivity was attempted.

In addition to autoradiography, these researchers compared levels of trichloroethylene and the metabolites trichloroethanol and trichloroacetic acid in maternal plasma, urine, amniotic fluid and foetal homogenates from a separate group of mice exposed to a high concentration (not stated) of unlabelled trichloroethylene for 1 hour. Trichloroethylene was detected in amniotic fluid and maternal plasma. No analysis of foetal homogenates was carried out for trichloroethylene. Both metabolites were detected in maternal plasma, amniotic fluid and foetal homogenates. It is not clear if metabolites were transferred across the placenta or generated within the foetoplacental unit.

A smaller study has been conducted in rats (Savolainen et al., 1977, quoted in Davidson and Beliles, 1991) in which animals were exposed to 200 ppm trichloroethylene for 6 hours per day, on 4 consecutive days. Rats were sacrificed after 0, 2, 3, 4 or 6 hours exposure on the fifth day and levels of trichloroethylene determined in the cerebellum, cerebrum, lungs, liver, perirenal fat and blood. Seventeen hours after exposure on day 4 (immediately prior to exposure on day 5), high levels of trichloroethylene were found in perirenal fat (0.23 nmol/g tissue) and blood (0.35 nmol/g tissue) but trichloroethylene was not detected in other tissues. This gives further evidence that trichloroethylene accumulates in fatty tissue. Levels of trichloroethylene in all tissues were more or less constant in rats sacrificed after 2 or more hours exposure on day 5 indicating that steady state had been reached within 2-3 hours. From the results, Savolainen et al. also calculated blood/tissue partition coefficients for these tissues. Values were 10.4 for adipose tissue/blood, 1.3 for brain/blood, 0.8 for lungs/blood, and 0.05 for liver/blood although this last figure is likely to be much higher owing to metabolic elimination of trichloroethylene from the liver. These partition coefficients clearly demonstrate that trichloroethylene will preferentially partition into fat-rich tissues.

In an earlier study it was found that in sheep and goats, levels of trichloroethylene in foetal blood taken from the umbilical vein were similar to those in maternal blood, taken from the carotid artery, within 15 minutes of the start of exposure (Helliwell and Hutton, 1950).

Trichloroethylene is therefore rapidly distributed throughout the body and readily crosses the blood-brain and placental barriers. It is also possible that the metabolites trichloroethanol and trichloroacetic acid may cross the placenta although it is also possible that these metabolites may have been generated within the foetoplacental unit. It has been observed that trichloroethylene preferentially partitions into lipid rich tissues and this would be expected given its high lipid solubility. In one rat study, a steady state for tissue distribution, including perirenal fat, was reached within 2-3 hours of the start of exposure. No differences in distribution were apparent between rats and mice.

Metabolism and elimination

Most studies on the metabolism of trichloroethylene have been conducted by the oral route (a schematic metabolic pathway for trichloroethylene is given at **Figure 1** and discussed under oral dosing). However, it is also possible to draw some conclusions about the extent to which

trichloroethylene is metabolised and routes of elimination for trichloroethylene and its metabolites from inhalation data.

In a study to examine the kinetics of trichloroethylene, groups of 16 male B6C3F1 mice or Osborne-Mendel rats were exposed (whole body) for 6 hours to either 10 or 600 ppm (54 or 3,228 mg/m^3) 14C-trichloroethylene (Stott et al., 1982). At the end of the exposure period, 4 animals from each group were placed in metabolism cages for the next 50 hours for collection of faeces, urine and exhaled air. The remaining 12 animals from each group were killed at 0, 6 or 24 hours post exposure and livers and kidneys removed to determine the amount of radioactivity bound to these tissues. Tissue samples for binding investigations were also taken from the animals in metabolism cages at the end of the 50-hour period.

Results showed that in male B6C3F1 mice the proportion of radioactivity metabolised and eliminated at both dose levels were similar. Around 9% of the total body burden was eliminated as $^{14}CO_2$, around 70% was eliminated as metabolites in the urine, around 4% in the faeces and only small differences were noted in the amount of unchanged ^{14}C-trichloroethylene exhaled. Between 97-99% of the absorbed dose was metabolised. In contrast, marked dose-related differences were found in male Osborne-Mendel rats. At 10 ppm, 5% of total body burden was eliminated as $^{14}CO_2$, 63% was eliminated in the urine and 7% in the faeces with 2% eliminated as unchanged ^{14}C-trichloroethylene, indicating metabolism of 98% of the total body burden. However, in rats exposed to 600 ppm, 3% of total body burden was eliminated as $^{14}CO_2$, 55% in the urine, 4% in the faeces and 21% as unchanged ^{14}C-trichloroethylene indicating that only 79% of the dose had been metabolised. Therefore while no evidence of metabolic saturation was found in male B6C3F1 mice, there was clear evidence of saturation in the male Osborne-Mendel rat.

Species differences were also noted in macromolecular binding in both hepatic and renal tissues and as with metabolism the differences were clearly related to dose. At 10 ppm, binding of radioactivity in the liver and kidney was similar in both species, but at 600 ppm, compared with the rat, four times as much radioactivity was bound in mouse liver and three times as much in mouse kidney. This difference was thought due to saturation of metabolism of trichloroethylene in the rat at the higher dose level. In both species, maximum binding was found to occur immediately post exposure in the liver and 3 hours post exposure in the kidneys.

Other groups have investigated the effects of compounds sharing metabolic pathways with trichloroethylene on the kinetics of this substance. Most attention has been focused on ethanol, a substance that seems to have a dual effect on the metabolism of trichloroethylene. Jakobson et al. (1986) reported that 0.8 ml/kg ethanol administered orally shortly before exposure to trichloroethylene inhibited the metabolism of trichloroethylene in rats particularly at lower exposure levels (50 and 100 ppm) giving rise to elevated levels of trichloroethylene in the blood. In contrast, high levels of ethanol (2 g per rat) ingested daily for 3 weeks enhanced the metabolism of trichloroethylene to trichloroacetic acid particularly at higher dose levels (500 and 1,000 ppm) (Kaneko et al., 1994). No effect on trichloroethylene metabolism was found in this study when rats were exposed to 50 or 100 ppm. Metabolic induction was also seen in rats given a single large oral dose of ethanol, 4 g/kg, around 15-18 hours prior to exposure to 400 ppm trichloroethylene (Sato et al., 1981, cited in Davidson and Beliles, 1991).

Other substances which have been shown to competitively inhibit the metabolism of trichloroethylene include 1,1,1-trichloroethane (Savolainen, 1981) and also tetrachloroethylene, isopropanol, pyrazole and tetraethylthiuram disulphide (Jakobson et al., 1986). Co-exposure to chloral hydrate had no effect indicating that the rate limiting step in trichloroethylene metabolism occurs during its conversion to chloral hydrate (Jakobson et al., 1986).

Earlier work showed that repeated exposures to trichloroethylene itself at occupationally relevant levels (50 or 100 ppm for 8 hours per day over 14 days) did not induce the metabolism of trichloroethylene in rats or hamsters (Ikeda and Immamura, 1973).

Summary of inhalation toxicokinetics

To summarise inhalation toxicokinetics in animals, trichloroethylene is readily absorbed by this route. Once absorbed it is rapidly distributed throughout the body readily crossing the blood-brain and placental barriers. Due to its high lipophilicity trichloroethylene has a tendency to accumulate in fatty tissues though in one repeated exposure study, steady state was reached within 2-3 hours, indicating that significant bioaccumulation of the parent compound is not likely to occur. There is evidence to show that metabolism of trichloroethylene is saturable at much lower levels in rats than in mice. Furthermore there is evidence that co-exposure to other compounds sharing metabolic pathways can have a significant effect on trichloroethylene blood levels indicating the importance of metabolism in the clearance of trichloroethylene from the body, although trichloroethylene itself does not seem to induce enzymes responsible for its metabolism. Elimination of trichloroethylene at low doses occurs predominantly as metabolites in the urine although at higher dose levels when metabolism becomes saturated elimination of unchanged trichloroethylene in exhaled air becomes more marked. This was particularly evident in rats where a 10-fold increase in elimination of unchanged trichloroethylene occurred when airborne concentrations of trichloroethylene were increased from 10 to 600 ppm. Other routes of elimination that have been identified include the faeces presumably via the bile and as CO_2 in exhaled air.

Oral

Absorption

Trichloroethylene is also well absorbed following administration by the oral route. It has been demonstrated that following oral dosing of radiolabelled trichloroethylene to rats, mice and rabbits, 80-98% of the radioactivity is recovered in expired air and urine (Prout et al., 1985; Dekant et al., 1984; Ogata and Saeki, 1974; Daniel, 1963). Dose levels in these studies ranged from 40-2,000 mg/kg. There is some evidence that uptake kinetics may differ between rats and mice. Larson and Bull (1992a) gave groups of 5-6 rats around 200, 600 and 3,000 mg/kg trichloroethylene and 5-6 mice around 200, 600 and 2,000 mg/kg trichloroethylene in aqueous Tween 80 and followed blood concentrations over time. In both species, peak blood concentrations were linearly related to dose. However, levels of trichloroethylene in mice rose much more rapidly than in rats and reached a much higher peak so that in mice, for a dose of 600 mg/kg, peak levels of 1,100 nmol/ml (150 μg/ml) were recorded 15 minutes post dosing (the first sample time) whereas in rats peak levels of 250 nmol/ml (32 μg/ml) were not reached until 2 hours post dosing. It was also apparent that whereas in mice, the 15-minute sample always contained the highest concentration of trichloroethylene, in rats, the time to reach peak concentrations increased with increasing dose. The basis for this apparent species difference in uptake is unclear.

Uptake of trichloroethylene has also been examined briefly in male F344 rats and male beagle dogs (Templin et al., 1995a). Groups of 4 rats were given 20 or 100 mg/kg trichloroethylene in 2% aqueous Tween 80 and groups of 4 beagles 20, 50 or 100 mg/kg in 2% aqueous Tween 80. Peak blood levels in both species increased with increasing dose. In the rat, the greatest blood concentration of trichloroethylene was recorded at the 0.75 hour time point and in the dog at the 1 hour time point. However, peak levels in the rat tended to be lower than those recorded from

the dog. This was most noticeable at the top dose, 100 mg/kg. In the rat, peak blood levels of around 80 nmol/ml (11 μg/ml) were recorded whereas in the dog levels of around 200 nmol/ml (27 μg/ml) were obtained. This result again suggests that oral uptake of trichloroethylene in the rat may be lower than in other species but the basis for this is unclear.

There is evidence that the vehicle in which trichloroethylene is given can affect the rate and extent of uptake. Withey et al. (1983) gave 18 mg/kg trichloroethylene in aqueous solution or corn oil to fasting male Wistar rats. For aqueous solutions, peak blood concentrations of around 15 μg/ml were reached within 6 minutes. Blood trichloroethylene levels were too low to be detectable following administration of 18 mg/kg in corn oil. However, data gathered from experiments in which higher concentrations had been dosed, between 50 and 2,500 mg/kg, indicated that peak blood levels of less than 1 μg/ml would be expected. Also whereas blood concentrations rapidly reached a single peak following dosing in aqueous solution, two peaks were observed following dosing in corn oil with the first peak occurring shortly after dosing and the second peak occurring after around 80 minutes. Therefore peak blood concentrations for trichloroethylene given in aqueous solution were much higher and reached much more rapidly than when trichloroethylene was given in corn oil. In addition, the area under curve ratios for the two solutions after 5 hrs were 218:1 water:corn oil giving further evidence that the absorption kinetics of trichloroethylene in aqueous solution are different to those of trichloroethylene in corn oil. It was suggested that the corn oil might be acting as a slow release vehicle for trichloroethylene. It is not clear how the different vehicle effects might alter metabolism and saturability.

Distribution

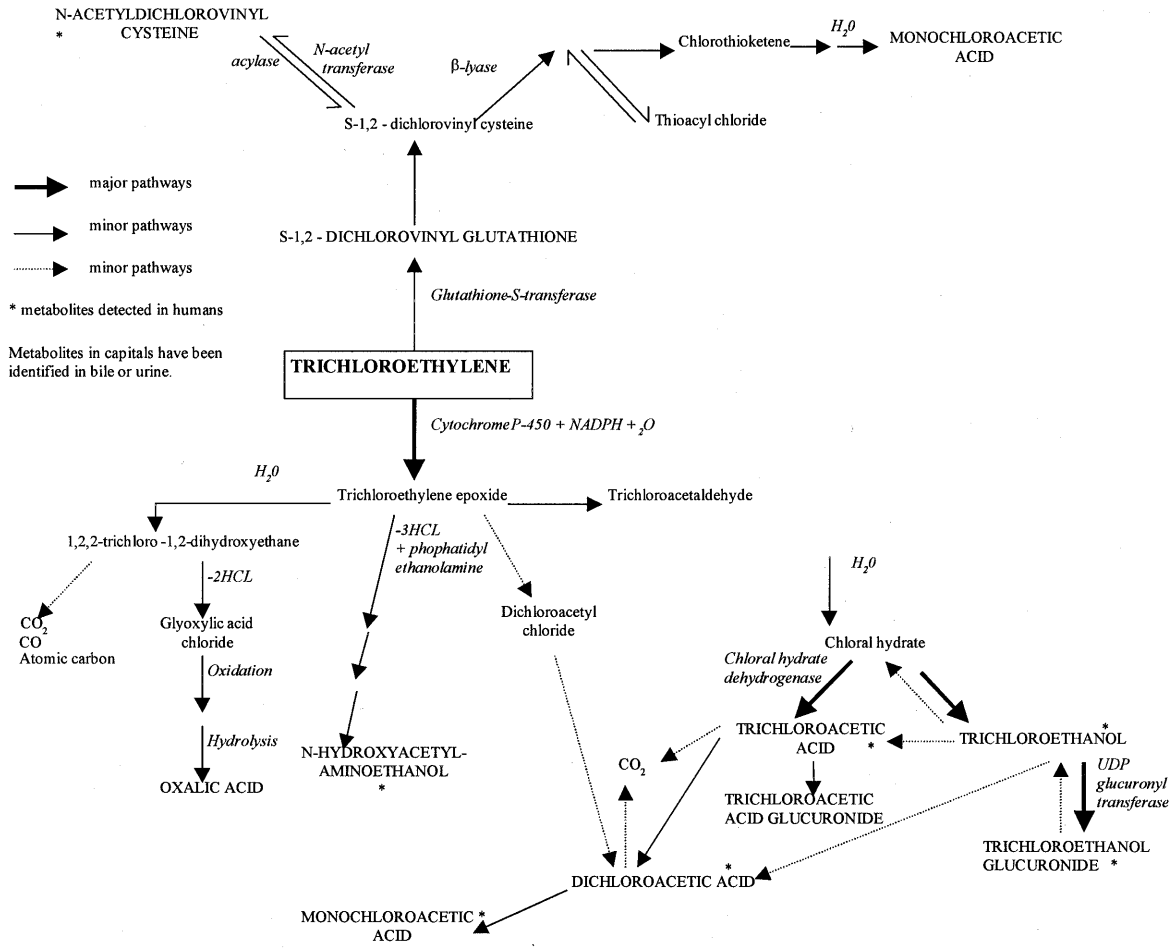
The distribution of trichloroethylene following repeated oral administration of 10-1,000 mg/kg for 6 weeks has been studied in Long Evans rats (Zenick et al., 1984). Levels of trichloroethylene and its metabolites trichloroethanol and trichloroacetic acid were determined in blood and tissues 4-5 hours after the last dose. As was found in inhalation studies, trichloroethylene distributed throughout the body with the highest levels occurring in fatty tissues. Trichloroacetic acid and trichloroethanol were also found throughout the body including the brain and reproductive organs. It was not possible to determine whether the metabolites were formed within the organs in which they were found or whether they had been formed elsewhere and were taken up from the blood.

Widespread distribution of trichloroethylene and its metabolites was also observed in rats and mice by Prout et al. (1985). Male Osborne-Mendel and Alderly-Park (Wistar derived) rats were given a single oral dose of 10 or 1,000 mg/kg 14C-trichloroethylene and male B6C3F1 and Swiss-Webster mice were given a single oral dose of 50 or 1,000 mg/kg ^{14}C-trichloroethylene. Autoradiograms taken three days later revealed radioactivity had distributed throughout the carcass with maximum concentrations in the liver.

Metabolism and elimination

The metabolism of trichloroethylene has been extensively studied in rats and mice following oral administration. Other species in which the toxicokinetics of trichloroethylene by the oral route has been studied in less detail include dogs, guinea pigs, hamsters and rabbits and it is apparent that the major metabolic pathways for trichloroethylene are the same between different species. A schematic metabolic pathway for trichloroethylene is included giving details of major and minor pathways (see **Figure 1**).

Figure 1. Schematic metabolic pathway for trichloroethylene



The initial step in the biotransformation of trichloroethylene is a rapid conversion by cytochrome P-450 to a transient metabolite trichloroethylene oxide. This epoxide is thought to undergo intramolecular rearrangement to form trichloroacetaldehyde, which is then hydrolysed to chloral hydrate. This acts as a substrate for the enzymes alcohol dehydrogenase and chloral hydrate dehydrogenase producing trichloroethanol and trichloroacetic acid respectively, which are excreted in the urine, the former mainly as the glucuronide and the latter as the free acid (Daniel, 1963; Butler, 1949; Ikeda et al., 1980; Bonse et al., 1975; Byington and Liebman, 1965; Costa et al., 1980; Liebman, 1965; Uehleke et al., 1977; Miller and Guengerich 1983). Müller et al. (1982) state that trichloroacetic acid-glucuronide has been isolated from chimpanzee urine and a trichloroacetic acid-coenzyme-A conjugate has been found in mouse faeces and bile (Green et al., 1984, cited in Davidson and Beliles, 1991). Other minor metabolites include carbon monoxide, carbon dioxide, monochloroacetic acid, dichloroacetic acid, oxalic acid, N-(hydroxyacetyl)-aminoethanol, S(1,2-dichlorovinyl) glutathione, N-acetyl dichlorovinyl cysteine and chloroform (Ogata and Saeki, 1974; Traylor et al., 1977; Hathaway, 1980; Dekant and Henschler, 1983; Dekant et al., 1984; Green and Prout, 1985; Dekant et al., 1986a; Bruckner, 1989; Dekant et al., 1990; Green et al., 1990; Birner et al., 1993, Bernauer et al., 1996; Green et al., 1997). Of these metabolites, the occurrence of oxalic acid, N-(hydroxyacetyl)-aminoethanol, the trichloroacetic acid conjugates with glucuronide and coenzyme-A have not been confirmed by more than one group of researchers. Although the major metabolic pathways are the same between different species, the major metabolite in all species studied so far being

trichloroethanol-glucuronide, there are marked quantitative differences between some species and strains. This has been investigated most thoroughly in rats and mice.

Recently extensive studies have been carried out investigating the kinetics of trichloroethylene and its metabolites trichloroethanol, trichloroacetic acid and dichloroacetic acid in male Sprague-Dawley rats and male B6C3F1 mice (Larson and Bull, 1992a; Bull et al., 1993). Groups of 5-6 rats were given a single oral dose of 200, 600 or 3,000 mg/kg trichloroethylene in 1% aqueous Tween 80. Blood samples were then collected at 1, 2, 4, 8, 12, 24, 48 and 72 hours post exposure. Groups of 5-6 male mice were given 200, 600 or 2,000 mg/kg trichloroethylene in 1% aqueous Tween 80 and sacrificed 0.25, 0.75, 2, 4, 8, 24, 48 or 72 hours later for collection of blood.

Clear differences in the metabolism of trichloroethylene were observed between rats and mice. In the rat, saturation of metabolism was apparent with doses of 600 mg/kg or more. This was reflected in the half-lives determined for trichloroethylene in the blood at each dose level. While trichloroethylene had a half-life of around 1.3 hours at 200 mg/kg, this increased to 3 hours at 600 mg/kg and 4.3 hours at 3,000 mg/kg. Dose dependency was also apparent in the rates at which the metabolites trichloroethanol and trichloroacetic acid were formed, with peak metabolite levels occurring at later times for higher doses. For trichloroethanol, peak levels at the low dose were recorded 2-3 hours after dosing whereas peak levels at the mid and high doses were reached after 8 and 12 hours respectively. The half-life of this metabolite in the blood was 2.0, 4.1 and 5.3 hours at the low, mid and high doses respectively. For trichloroacetic acid, at the low dose, peak levels were reached between 4 and 8 hours post dosing whereas peak levels at the mid and high doses were reached after 12 and 24 hours respectively. The half-life of this metabolite in the blood was 5.0, 7.0 and 7.0 hours at the low, mid and high doses respectively. There is therefore clear evidence of saturation of metabolism in the rat.

In mice, there was some evidence of metabolic saturation. The rate of clearance of trichloroethylene from the blood showed a slight dose dependency with half-lives ranging from 0.5 hours at 200 mg/kg to 0.8 hours at 600 mg/kg and 1.1 hours at 2,000 mg/kg. There were also dose-related differences in the rates of formation of trichloroethanol and trichloroacetic acid. However, rates of formation for both trichloroethanol and trichloroacetic acid in mice were always around twice those determined for rats showing that mice metabolise trichloroethylene faster than rats.

For trichloroethanol, peak levels at all doses were reached within 2 hours in mice and levels of free trichloroethanol were 10 times greater in mice than those in rats for an equivalent dose of trichloroethylene. The half-life of trichloroethanol in mice was 0.5-0.7 hours at the low and mid dose but increased to 2.7 hours at the top dose. For trichloroacetic acid, peak levels were reached after 1 hour at the low dose but only after 8 hours at the mid and high doses. Peak levels of trichloroacetic acid in mice were two and a half times those in rats at the low dose but four times those in rats at the mid dose. The half-life of trichloroacetic acid in mice was 4-5 hours at the low and mid dose but increased to 7.7 hours at the top dose.

Although differences were apparent in the rates of metabolism and elimination of trichloroethylene, areas under the curve for trichloroethylene were very similar for rats and mice showing both species absorbed similar amounts of trichloroethylene. In contrast areas under the curve for the metabolites trichloroethanol and trichloroacetic acid were 1.5 to 2.5 times greater in mice than rats at all dose levels showing that mice metabolised a greater portion of the dose than did rats.

In addition to trichloroethanol and trichloroacetic acid, the metabolite dichloroacetic acid was also studied. Results showed that dichloroacetic acid levels were only quantifiable in blood from

mice given 2,000 mg/kg. At this dose level, dichloroacetic acid was reported to have a rate of formation slightly greater than that of trichloroacetic acid at the same dose level and a half-life of 4.6 hours. At lower doses in mice dichloroacetic acid was detectable but in insufficient amounts to measure. Dichloroacetic acid was not detected (detection limit was 4 nmol/ml, around 5 μg/ml) in rats at any dose level.

The kinetics of trichloroacetic acid and dichloroacetic acid formation in male B6C3F1 mice have been further characterised by this group (Templin et al., 1993). Mice were given around 20, 50, 100, 500 or 2,000 mg/kg trichloroethylene in 2% aqueous Tween 80 and blood samples taken 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 9, 12, 18, and 36 hours after dosing for determination of trichloroethylene, trichloroethanol, trichloroacetic acid and dichloroacetic acid. Levels of trichloroacetic acid in liver homogenates prepared from orally dosed mice were also determined and in a separate group of mice given ^{14}C-trichloroethylene, studies of plasma protein binding of trichloroacetic acid were also undertaken.

The kinetics of trichloroethylene, trichloroethanol and trichloroacetic acid in the blood were consistent with those reported from the earlier studies. In addition, this further work revealed that concentrations of free trichloroethanol doubled between dose levels of 100 and 500 mg/kg suggesting saturation of trichloroethanol glucuronidation in mice at the higher dose levels. It was found that dichloroacetic acid could be quantified in blood from mice given 100 mg/kg trichloroethylene or more. When kinetic parameters were calculated it became apparent that like trichloroethanol and trichloroacetic acid, the rate of formation of dichloroacetic acid was dose dependent, with peak metabolite levels occurring at later times for higher doses. Peak levels were reached after 1.5 hours following doses of 100 or 500 mg/kg trichloroethylene increasing to 4 hours following a dose of 2,000 mg/kg. It was found that although the actual peak levels were similar at these dose levels, the area under the curve increased linearly with dose. This was reflected in the half-lives calculated for dichloroacetic acid. At 100 mg/kg trichloroethylene a half-life of 0.5 hours was calculated in comparison to 2.6 hours at the two higher dose levels. In addition, the area under the curve for dichloroacetic acid was found to be greater than that predicted if dichloroacetic acid was formed solely from trichloroacetic acid suggesting the possibility of an additional metabolic pathway for the formation of dichloroacetic acid. Recently evidence that dichloroacetic acid may be generated from trichloroacetic acid during sample preparation has come to light (Templin et al., 1995b). As yet there are no data on the extent to which this occurs, therefore it is not possible to draw any conclusions regarding the extent to which mice metabolise trichloroethylene to dichloroacetic acid. However, given that no dichloroacetic acid was detected in blood from other species prepared for analysis using the same technique this supports the conclusion that dichloroacetic acid is a metabolite of trichloroethylene in mice.

Further investigations into the blood and tissue kinetics of trichloroacetic acid revealed that trichloroacetic acid partitioned into the blood of male B6C3F1 mice in preference to the liver. It was suggested that binding to plasma proteins could account for this. Studies of plasma binding of trichloroacetic acid indicated that at low blood trichloroacetic acid concentrations (below 50 μg/ml) at least 50% of the trichloroacetic acid in blood is bound to plasma proteins. At higher concentrations however the proportion of bound trichloroacetic acid decreases so that around 41, 34 and 23% of trichloroacetic acid was bound at blood concentrations of 50, 100 and 200 μg/ml respectively.

Lately these studies have been extended to compare the kinetics of trichloroethylene and its metabolites in the F344 rat and beagle dog (Templin et al., 1995a). Groups of 4 male rats and 4 male dogs were given single oral doses of 20 or 100 mg/kg trichloroethylene in 2% aqueous

Tween 80; an additional 50 mg/kg dose group was included for dogs. Serial blood samples were collected from rats between 0.25 and 48 hours post dosing and from dogs between 0.25 and 240 hours. Additional experiments were performed to briefly investigate urinary elimination in the dog and biliary elimination in the rat and the extent of plasma binding of trichloroacetic acid to plasma proteins was investigated for rat, dog and human plasma *in vitro*.

Results of the kinetic studies revealed some differences between rats and dogs in the metabolism and elimination of trichloroethylene and its metabolites. Also, comparing the results obtained for the rat at these dose levels to those obtained by this group in earlier studies at higher dose levels (Larson and Bull, 1992a; Bull et al., 1993) some inconsistencies were apparent. While rates of formation for metabolites, peak concentrations, half lives and areas under the concentration-time curve followed the same dose-related trends, the values obtained in the present studies were often higher than would be predicted from the earlier results. The reasons for these inconsistencies are unclear.

In the present investigation, the half-lives of trichloroethylene in the rat following doses of 20 or 100 mg/kg were 0.15 and 0.4 hours respectively. For rats given 100 mg/kg trichloroethylene, peak trichloroethanol levels were recorded at around 2.5 hours post dosing but the half-life of this metabolite was determined to be 5.5 hours, longer than would be predicted from the previous study. It was stated that at the 12 hour collection point, trichloroethanol-glucuronide accounted for 63% of the total trichloroethanol levels. Trichloroethanol was not measured in the low-dose group for rats. As before there was evidence of dose-dependency in the rate of formation of trichloroacetic acid. Peak concentrations of this metabolite were recorded around 12 hours post dosing and the half lives following doses of 20 or 100 mg/kg trichloroethylene were around 8 and 13 hours respectively. Again these are longer than would be predicted from the previous study. Dichloroacetic acid was not detected at any time point. The limit of detection was 5μg/ml.

In the dog, the half-lives for trichloroethylene in the blood were similar to those obtained in the rat at equivalent dose levels. For doses of 20, 50 or 100 mg/kg trichloroethylene, half lives of 0.25, 0.36 and 0.57 hours respectively were determined. The rate of formation of trichloroethanol remained constant across this dose range and as was the case in the rat, peak levels of trichloroethanol were recorded around 2.5 hours post dosing. Analysis of blood samples taken 12 hours post dosing revealed that 80% of total trichloroethanol was present as the glucuronide. This is similar to the mouse in which species 83% of total trichloroethanol was reported to be glucuronidated. The mouse data were obtained from earlier studies (Templin et al., 1993). Dose-dependency was apparent in the rate of formation of trichloroacetic acid. Peak blood levels were reported to occur around 24 hours post dosing. In the dog, trichloroacetic acid was found to have a half-life of around 200 hours and in contrast to findings in the rat, there was no dose-dependency for the half-life of trichloroacetic acid in the blood across this dose range.

In addition to the blood kinetics studies, urinary elimination of trichloroethanol and trichloroacetic acid were followed in the dog. The half-life for urinary trichloroethanol was around 12 hours. It was found that 80% was eliminated within the first 48 hours post dosing, with peak elimination occurring between 24 and 48 hours. Most trichloroethanol recovered from the urine was in the form of the glucuronide. During the first 24 hours around 28% was eliminated as free trichloroethanol but in later collections this declined to less than 1% of the total urinary trichloroethanol. The half-life for urinary trichloroacetic acid in the dog was determined to be around 205 hours closely matching the half-life for this metabolite in the blood. Results from brief analyses of rat bile indicated that by this route, trichloroethanol was predominantly eliminated as the glucuronide.

The extent to which trichloroacetic acid bound to plasma constituents was studied using concentrations of 1, 10 or 100 μg/ml trichloroacetic acid in rat, dog and human plasma. Samples were obtained from groups of 4 male rats, dogs or humans. Results showed that in all species the extent to which trichloroacetic acid was bound to plasma proteins decreased with increasing dose. In human plasma, between 85, 83 and 75% of trichloroacetic acid was bound at these respective concentrations. Using dog plasma binding decreased from 65 to 54% across the range and with rat plasma, a decrease from 54 to 38% was observed. Comparing these results to those previously reported for mice it can be seen that of the species studied so far, mice have the least capacity for plasma binding of trichloroacetic acid.

The routes and kinetics of elimination have also been compared between two strains of rat (Osborne-Mendel and Wistar) and two strains of mouse (B6C3F1 and Swiss-Webster) following single oral doses of between 10 and 2,000 mg/kg 14C-trichloroethylene in corn oil (Prout et al., 1985; Green and Prout, 1985). Animals were housed in metabolism cages for 72 hours following dosing for collection of urine, faeces and exhaled air. The percentage of the dose eliminated by these routes was then calculated and analysis carried out to determine which compounds were present.

At the lowest dose level, no significant differences were seen between rats and mice in the percentage of dose eliminated either in exhaled air or as metabolites in the urine and faeces. In total around 65% of the dose was recovered as metabolites in the urine, around 7-23% as metabolites from the faeces, 9-13% was eliminated as CO_2, and 1-4% as unchanged trichloroethylene in exhaled air. All unchanged trichloroethylene was eliminated on the first day and total recovery was 96-100% of the administered dose.

At higher dose levels, only slight changes in routes of elimination were found in mice. At 2,000 mg/kg, 48% of the administered radioactivity was recovered as metabolites in urine and 14% as trichloroethylene in exhaled air. In contrast, in rats at all higher dose levels (500, 1,000 and 2,000 mg/kg), dose related changes in routes of elimination were apparent. This effect was most marked at the top dose where the percentage of the dose eliminated in urine fell markedly (to around 14%) while the proportion of administered trichloroethylene eliminated unchanged in exhaled air rose to around 78%. Less marked reductions were seen in the percentage of the dose eliminated in faeces and as CO_2 with increasing dose, and proportionately less radioactivity remained in the carcass.

Detailed analyses of eliminated radioactivity revealed that the same metabolites were formed in all strains of rats and mice included in this experiment. Although the proportion of the administered dose of ^{14}C-trichloroethylene eliminated via a given route varied according to species and dose level, only minor differences were observed between the species in the ratios of metabolites eliminated via a given route. Hence, however much of the administered dose was eliminated in expired air, the majority of radiolabel eliminated in both rats and mice was accounted for by trichloroethylene and CO_2 with trichloroethanol and CO accounting for <0.1% each. Similarly, in both rats and mice, the major urinary metabolite was trichloroethanol-glucuronide accounting for around 88% of the urinary radioactivity. Trichloroacetic acid accounted for a further 6-8% of the urinary radioactivity and 2% was accounted for as free trichloroethanol; dichloroacetic acid was found to account for <1% and monochloroacetic acid accounted for <0.1%. In addition to urine, bile samples were collected from rats and mice given 500 mg/kg. It was reported that in rats, 10% of the administered radioactivity was eliminated in the bile and 26% in the urine at this dose level. Of the radioactivity eliminated in the bile, 13.5% was trichloroacetic acid, 63% trichloroethanolglucuronide and 3% free trichloroethanol. A further 15% could not be identified. The proportion of the administered radioactivity eliminated in the bile in mice was not reported. However, the percentage of biliary radioactivity eliminated

as trichloroacetic acid and trichloroethanol-glucuronide in mice were similar to those found in rat bile, but the unknown fraction represented only 2.6% of the total biliary radioactivity.

In a more recent study, conducted as part of an investigation into trichloroethylene induced nephrotoxicity, male F344 rats were given 500 or 2,000 mg/kg trichloroethylene for 1 or 10 days with 14C-trichloroethylene incorporated into the dose on days 1 and 10 (Green et al., 1990). Urine was collected for 24 hours following the final dose for determination of trichloroacetic acid and N-acetyl dichlorovinyl cysteine levels (N-acetyl DCVC). Results showed that trichloroacetic acid accounted for around 7% of the administered dose but N-acetyl DCVC only accounted for between 0.001 to 0.008% of the administered dose This study demonstrated the existence of a reductive, glutathione-conjugation metabolic pathway, but suggested that this is quantitatively a minor pathway in the rat. Further studies (Birner et al., 1993; Bernauer et al., 1996; Green et al., 1997) investigating the glutathione pathway are discussed in detail in the carcinogenicity section (Section 4.1.2.8).

Prout et al. (1985) also compared the kinetics of trichloroethylene and the major metabolites (chloral hydrate, trichloroethanol and trichloroacetic acid) in rats and mice given a single dose of 1,000 mg/kg trichloroethylene in corn oil. Blood concentration versus time curves showed that kinetics observed in these strains of rats and mice were consistent with those observed by other workers in Sprague-Dawley rats and B6C3F1 mice (Larson and Bull, 1992a). Measurements were only taken from one animal per time point in this study so it was only possible to make general comparisons between the two species. These results support the conclusion that mice metabolise trichloroethylene more rapidly and more extensively than do rats.

Dekant et al. (1984) compared routes of elimination between Wistar rats and NMRI mice and identified which metabolites had been eliminated. Seventy two hours after a single gavage dose of 200 mg/kg ^{14}C-trichloroethylene in corn oil, to female Wistar rats, 54% was recovered from the exhaled air of which 52% was trichloroethylene and 2% CO_2. A further 41% was recovered from the urine with 2% in the faeces and 3% in the carcass. In contrast in NMRI mice, 17% was eliminated in the air of which only 11% was trichloroethylene and 6% CO_2. A further 76% was recovered from the urine with 5% in the faeces and 2% in the carcass. These results are broadly similar to those obtained by Prout et al. (1985) above and give further evidence that metabolism in rats is much more readily saturated than in mice. Dekant et al. (1984) also determined which metabolites were present. Trichloroethylene and CO_2 were the only metabolites detected in exhaled air. Again, the major metabolite in both species was trichloroethanol. In rats, 62% of urinary radioactivity was accounted for by conjugated trichloroethanol and 12% as free trichloroethanol. A further 15% was found to be trichloroacetic acid and 2% was dichloroacetic acid. N-(hydroxyacetyl)-aminoethanol accounted for 7% and oxalic acid 1% of urinary radioactivity. In mice, 94% of urinary radioactivity was accounted for by conjugated trichloroethanol with only 0.1% free trichloroethanol. In contrast to the strains of mice investigated by Green and Prout (1985) trichloroacetic acid accounted for only 0.1% of the total radioactivity. Other metabolites identified were dichloroacetic acid 0.1%, N-(hydroxyacetyl)-aminoethanol 4% and oxalic acid 0.7%.

Mitoma et al. (1985) compared the metabolic disposition of trichloroethylene between rats and mice given the maximum tolerated dose (MTD) (1,300 mg/kg in rats and 2,000 mg/kg in mice) and 1/4 of the MTD used in the NCI carcinogenicity bioassay (see carcinogenicity section for details). Unlabelled trichloroethylene in corn oil was given at both dose levels for 5 days/week over 4 weeks followed by a single dose of radiolabelled trichloroethylene. Animals were then housed in metabolism cages for 48 hours for collection of exhaled air, urine and faeces. Measurements were also made of radioactivity remaining in the carcass and of radioactivity bound to proteins in the liver.

The results of the metabolism studies showed that although rats received a lower dose of trichloroethylene, at the MTD they eliminated substantially more unchanged trichloroethylene (57%) than did mice (18%). No results were presented for animals given 1/4 the MTD. This study gives further evidence that at the higher dose levels used in the NCI cancer bioassays mice were metabolising considerably more of the dose than rats. Species differences in protein binding were also apparent, again reflecting saturation of metabolism in rats.

In addition to their single dose studies, Green and Prout (1985) also investigated the effects in mice of repeated dosing of 1,000 mg/kg 7 days/week for 10 or 180 days to see if any induction of metabolism could be occurring in this species. It was found that the proportion of parent compound that was metabolised remained the same but that the fraction that was trichloroacetic acid doubled from 10 to 20% of the urinary radioactivity and the fraction of trichloroethanol dropped from 88 to 77% to compensate. This effect was evident after 10 days and no further changes were apparent after 180 days. This may reflect an increase in oxidative metabolism to trichloroacetic acid at the expense of reductive metabolism to trichloroethanol over the course of repeated dosing or may be because trichloroacetic acid has a long half-life in the body and does not reach steady state for several days because of its long half-life. No evidence of metabolic induction was found.

Summary of oral toxicokinetics

To summarise oral toxicokinetics in animals, trichloroethylene is well absorbed by the oral route and distributes throughout the body. There are no significant differences in routes of elimination following oral or inhalation dosing, therefore it is likely that metabolism will be the same for each route of exposure. The major metabolic pathway in all species studied so far involves conversion of trichloroethylene by cytochrome P450 to from an epoxide. This rearranges to form mainly trichloroacetaldehyde, which is further metabolised to yield trichloroethanol glucuronide and trichloroacetic acid. Other minor metabolites which have been recovered either from exhaled air or urine include carbon dioxide and monoxide, free trichloroethanol, trichloroacetic acid conjugates with glutathione and coenzyme A, oxalic acid, N-hydroxyacetyl aminoethanol, dichloroacetic acid and monochloroacetic acid. There is also evidence that a small fraction of trichloroethylene is conjugated with glutathione. Evidence for this comes from the isolation of S(1,2-dichlorovinyl) glutathione in the bile of rats and N-acetyl DCVC from the urine of rats and mice. It therefore seems likely that all species share common metabolic pathways for trichloroethylene.

However, there is convincing evidence that not all species metabolise trichloroethylene to the same extent. Comprehensive studies in rats and mice have shown that mice metabolise trichloroethylene more quickly and to a greater extent than do rats. In rats, metabolism of trichloroethylene is saturated at oral dose levels of 1,000 mg/kg whereas in mice, only slight reductions in the proportion of trichloroethylene which is metabolised are seen at dose levels of up to 2,000 mg/kg. While the proportion of the metabolised dose which passes down any given pathway seems to remain the same between rats and mice, the difference in saturability means that mice achieve higher peak levels and greater body burdens of all trichloroethylene metabolites than rats particularly at higher dose levels. There is also evidence that in mice trichloroethanol glucuronidation is saturated at a lower dose level than some other pathways. It has been shown in mice that trichloroacetic acid binds extensively to plasma proteins; this has not been investigated in other species.

Dermal

Dermal uptake of liquid trichloroethylene has been studied in guinea pigs and mice. In one study, the uptake of undiluted trichloroethylene was studied using 4 anaesthetised guinea pigs exposed for 6 hours to the substance in a sealed chamber (Jakobsen et al., 1982). Blood was collected at 5-20 minute intervals during exposure. Peak blood levels of around 1 μg/ml blood occurred after half an hour. Blood concentrations then decreased despite continuing exposure to the substance. After 6 hours exposure the blood level was reported to be 0.46 μg/ml. Blood levels were still declining at this time. The reason why blood trichloroethylene levels should decline despite continuing exposure is unclear but it may be because the rate of metabolism may be exceeding the rate of uptake. Alternatively, the rate of uptake from the external pool may slow as the concentration of trichloroethylene in a skin depot rises.

In an earlier study, male ICR mice were exposed to liquid trichloroethylene for 5, 10 or 15 minutes. Results showed that the amount of trichloroethylene absorbed increased linearly with time over this period. It was calculated that the rate of dermal absorption through mouse abdominal skin was around 8 $\mu g/cm^2$/minute (Tsuruta, 1978).

The uptake of trichloroethylene from dilute aqueous solutions has also been investigated (Bogen et al., 1992). Anaesthetised hairless euthymic guinea pigs were immersed in either a low (0.02-0.1 ppm) or high (100 ppm) concentration solution of ^{14}C-trichloroethylene for 70 minutes. The rate of uptake (permeability constant) was assessed from the rate of disappearance of radiolabel in the immersion solution and was found to be around 5.4 $\mu g/cm^2$/minute for both low and high concentration solutions. In addition, the elimination of radiolabel was followed in urine and faeces for 2-4 weeks after exposure. Around 59% of the estimated absorbed dose was eliminated and the time to eliminate 95% of the recovered radioactivity ranged from 7 to 14 days.

The results of these dermal studies suggest that both liquid trichloroethylene and trichloroethylene in aqueous solution is well absorbed. There are no data on the dermal uptake of trichloroethylene from the vapour phase. Although no studies of metabolism, distribution or elimination have been conducted in animals exposed to trichloroethylene by the dermal route, there is no evidence to suggest that it is different from that described for oral and inhalation exposures.

Studies *in vitro*

Most *in vitro* studies on trichloroethylene have been conducted to characterise metabolism in hepatic tissue. Relatively little is known about metabolism in extrahepatic tissues taken from different species and strains. Odum et al. (1992) investigated the metabolism of trichloroethylene in female CD-1 mouse lung Clara cells and compared this with metabolism in hepatocytes taken from these mice. Recovery of metabolites was reported to be greater than 95%. Results showed that Clara cells were able to metabolise trichloroethylene to chloral hydrate but further metabolism to trichloroethanol was slow and Clara cells in this strain of mouse were unable to form trichloroethanol conjugates with either glucuronide or sulphate. This resulted in a build up of primarily chloral hydrate within the Clara cells. In contrast, in hepatocytes, onwards metabolism of chloral hydrate proceeded much more readily so that the major metabolite was always trichloroethanol with trichloroethanol-glucuronide the second most abundant. Levels of chloral hydrate and trichloroacetic acid were always lower than levels of total trichloroethanol.

Metabolism of trichloroethylene has also been assessed in isolated rat and guinea pig lungs (strains not specified) perfused with whole blood (Dalbey and Bingham, 1978). Lungs were exposed to trichloroethylene in ventilation gas supplied to the lungs. In both species

trichloroethanol and trichloroacetic acid were detected in the perfusate but not chloral hydrate or trichloroethanol-glucuronide. Levels of trichloroethanol in the perfusate increased with time and guinea pigs consistently produced more trichloroethanol than did rats. Addition of ethanol to the blood used to perfuse the lungs did not affect the rate or extent of trichloroethanol formation. These two studies suggest that rat and guinea pig lungs are more efficient at onwards metabolism of chloral hydrate from trichloroethylene than mouse lung Clara cells although this has not been confirmed.

In a study investigating dermal uptake of trichloroethylene, Tsuruta (1978) determined a rate of uptake of trichloroethylene across SD-JCL rat skin *in vitro* to be around 12 μg/cm^2/minute.

4.1.2.1.2 Studies in humans

Inhalation

The toxicokinetics and metabolism of trichloroethylene have been extensively investigated in humans following exposure by the inhalation route. Information has been obtained from volunteer studies and also from those exposed occupationally and those exposed to the substance as an anaesthetic.

Absorption

There is a wide body of evidence to show that as is the case in animals, trichloroethylene is readily taken up across the lungs. Results from several studies indicate uptake of between 28 and 80% of the trichloroethylene in inspired air (Soucek and Vlachova, 1960; Bartonicek and Teisinger, 1962; Ahlmark and Forssman, 1951; Nomiyama and Nomiyama, 1974a, 1977; Fernandez et al., 1975; Astrand and Ovrum, 1976; Vesterberg et al., 1976; Monster et al., 1979). Typically, exposure levels ranged between 100 and 370 ppm for periods of 30 minutes to 5 hours, although in one study individuals were exposed for 10 minutes to up to 3,700 ppm. There was no evidence that percentage uptake was lower at higher airborne levels of trichloroethylene. In one study in which 5 volunteers were exposed to 100 ppm trichloroethylene for 6 hours, it was found that blood trichloroethylene levels during exposure increased steadily to reach a peak of around 1 μg/ml after 2 hours (Müller et al., 1974). Blood levels then stayed constant for the remainder of the exposure period. Sato and Nakajima (1978) reported mean blood trichloroethylene levels of 1.7 μg/ml immediately after a 4-hour exposure of 4 males to 100 ppm. Results from an earlier study showed peak blood levels of around 6 μg/ml also occurring after 2 hours exposure to 211 ppm (Stewart et al., 1962). However, levels of around only 4 μg/ml were found in the blood after three hours exposure. This suggests that in humans the blood and air reach equilibrium after around 2 hours continuous exposure, although it is expected that equilibrium with other tissues would take longer.

There is evidence to show that increasing workload increases the amount of trichloroethylene taken up at any given dose level. In one study, groups of 5 male volunteers aged between 22 and 29 years who had not consumed alcohol for 24 hours prior to the experiment were exposed for a total of four 30-minute periods over 140 minutes to either 100 or 200 ppm (540 and 1,080 mg/m^3) trichloroethylene via breathing apparatus (Astrand and Ovrum, 1976). During each 30-minute period, subjects were either at rest or were exercising at 50, 100 or 150 W intensities. Blood and expired air were sampled at regular intervals to determine trichloroethylene concentration both during and for two hours following exposure. Results showed that trichloroethylene uptake (defined as the difference in concentration between inspired air and

expired air) was around 55% for both exposure levels at rest but decreased during exercise such that only 25% of the inspired air concentration was retained during exercise at 150W. However, it was noted that although the percentage retention had fallen, the concentration of trichloroethylene in the blood increased during exercise because of the increased ventilation rate. Hence at rest, 30 minutes exposure to 100 ppm trichloroethylene gave rise to a mean arterial blood concentration of 1.3 μg/ml whereas 30 minutes exposure to 100 ppm during 50W exercise gave rise to arterial blood concentrations of around 3 μg/ml. Volunteers were allowed a 20-minute period free from exposure between exposure at rest and exposure during exercise. This trend was also observed in volunteers exposed to 200 ppm trichloroethylene.

Similar results were obtained by Monster et al. (1976), who found that the total amount of trichloroethylene taken up increased by around 40% in 4 male volunteers exposed to 70 or 140 ppm for 4 hours with two half hour periods of 100W exercise in comparison with 4 hours exposure at rest.

Distribution

The distribution of trichloroethylene in humans has not been studied experimentally. Its effectiveness as an anaesthetic indicates that it readily crosses the blood brain barrier. In one study of individuals admitted to an obstetrics department and anaesthetised with trichloroethylene during parturition, it was shown that within 15 minutes of the start of exposure, levels in foetal blood taken from the umbilical vein were similar to those in maternal blood taken from the vein of the left forearm, indicating rapid placental transfer (Laham, 1970). It can therefore be assumed that as is the case in animals, trichloroethylene is rapidly distributed throughout the human body.

Metabolism and elimination

After inhalation, between 10 and 28% of the trichloroethylene absorbed is excreted unchanged in the breath (Bartonicek, 1962; Nomiyama and Nomiyama, 1974a; 1974b; 1977; Morgan et al., 1970; Fernandez et al., 1975; Monster et al., 1976; 1979) and between 48 and 85% is eliminated in the urine as trichloroethanol and trichloroacetic acid (Nomiyama and Nomiyama, 1971; Fernandez et al., 1975; Müller, 1972; Monster 1976). Trichloroethanol and trichloroacetic acid have also been identified in the faeces, accounting for a further 8% of the dose (Bartonicek, 1962) and trichloroacetic acid, but not trichloroethanol, has been identified in bile (Ogata et al., 1988,). Trichloroethanol has also been identified in exhaled air at a level around 15,000 times lower than that found in blood, showing that this is a very minor route of elimination for this metabolite (Monster et al., 1976, 1979). Other metabolites that have been identified include chloral hydrate, identified in plasma (Cole et al., 1975), chloroform in the breath (Stewart et al., 1974; Souchek and Vlachova, 1955) and monochloroacetic acid, dichloroacetic acid, N-(Hydroxyacetyl)-aminoethanol and N-acetyl DCVC in urine (Stewart et al., 1974; Soucek and Vlachova, 1955, 1960; Hathaway, 1980; Dekant et al., 1984; Birner et al., 1993; Bernauer et al., 1996; Lash et al., 1999). The relative importance of these minor pathways has not been determined in humans. It therefore seems likely that the metabolic pathways in humans are qualitatively similar to those described in animals.

As is the case in animals, trichloroethanol seems to be the major urinary metabolite accounting for 29-50% of the absorbed trichloroethylene although the relative proportions of free trichloroethanol and trichloroethanol-glucuronide have not been determined (Soucek and Vlachova, 1960; Nomiyama and Nomiyama, 1971; Fernandez et al., 1975; Monster et al., 1976, 1979). Trichloroacetic acid was found to account for 10-24% of the absorbed trichloroethylene,

although the data of Nomiyama and Nomiyama (1971) indicate that trichloroacetic acid could account for up to 44%. In comparison, for the strains of rats and mice in which metabolites have been quantified, it was found that 76-90% of the absorbed dose was eliminated as urinary trichloroethanol and 6-15% as urinary trichloroacetic acid (Green and Prout, 1985; Dekant et al., 1984). Humans therefore seem to eliminate less of the absorbed dose as urinary trichloroethanol and more as urinary trichloroacetic acid than do rats and mice. In the only study in which monochloroacetic acid was quantified, urinary elimination of this metabolite accounted for 4% of the absorbed trichloroethylene (Soucek and Vlachova, 1960), which is substantially more than the <0.1% of the absorbed trichloroethylene eliminated as urinary monochloroacetic acid by certain strains of rats and mice (Green and Prout, 1985). The proportion of trichloroethylene metabolised by other pathways in humans was not determined.

Various groups have investigated the elimination kinetics of trichloroethylene and its major metabolites, trichloroethanol and trichloroacetic acid. Trichloroethylene is primarily removed from the body either by metabolism or via the lungs in exhaled air. In one study, elimination of unchanged trichloroethylene from the blood and breath was followed after a single 4-hour exposure of 4 males to 100 ppm (Sato et al., 1977). It was shown that the concentration of trichloroethylene in exhaled air was directly proportional to the concentration in the blood and that elimination from the tissues was best described by a three compartment model composed of richly perfused tissues (t½ = 2-3 minutes), lean body mass (t½ around 30 minutes) and fat-rich tissues (t½ = 3.5-5 hours). Similar patterns of elimination for trichloroethylene have been reported by Stewart et al. (1974), Müller et al. (1974), Fernandez et al. (1975) and Nomiyama and Nomiyama (1974a, 1974b).

Elimination of trichloroethylene has also been studied in 5 male volunteers exposed to 70-75 ppm for 4 hrs/day on 5 consecutive days (Monster et al., 1979). Again concentrations in exhaled air were directly proportional to those in blood. Although concentrations of trichloroethylene in the blood up to 100 minutes post exposure were similar on each day, blood concentrations 18 hours post exposure on day 5 were twice those after exposure on day 1 and this was thought to be due to slow release following partitioning of trichloroethylene which had accumulated in fat-rich tissues.

In one comprehensive series of investigations, the elimination of trichloroethanol and trichloroacetic acid from blood and in urine were characterised following both single and repeated exposures to trichloroethylene (Ertle et al., 1972; Müller et al., 1972; 1974). In the single exposure studies, a group of 5 male volunteers were exposed to 100 ppm trichloroethylene for 6 hours. The repeated exposure studies involved groups of 5-6 male volunteers who were either exposed to 50 or 100 ppm for 6 hours/day on 5 consecutive days or intermittently exposed to 250 ppm for 12 minutes/hour, 6 hours/day for 5 days. Blood and urine samples were taken regularly until elimination was nearly complete. Trichloroethanol was measured in whole blood and trichloroacetic acid in plasma. Throughout the experiments volunteers abstained from alcohol, tobacco and other drugs.

Results showed that during exposure to trichloroethylene, levels of trichloroethanol in blood rose steadily but did not reach a plateau within the 6-hour exposure period. Immediately post exposure, levels of trichloroethanol declined exponentially and it was calculated that the half-life for trichloroethanol in the blood was around 12 hours. As would be expected, given this half-life, there was some accumulation of trichloroethanol between exposures so that peak levels and the residual trichloroethanol concentration (that which had not yet been eliminated by the start of the next days exposure) increased over the 5 days exposure. For volunteers exposed to 50 ppm, peak blood levels of trichloroethanol had increased over the week from around 1.6 to 2 μg/ml, and following exposure to 100 ppm, peak blood levels had increased over the week from around 3.2

to 5 μg/ml. Increases in blood concentrations of trichloroethanol from 1.7 to 2.4 μg/ml were observed following intermittent exposure to 250 ppm. The greatest increase occurred between the first and second days and by the fifth day the system was approaching steady state. Initially peak concentrations of trichloroethanol in the blood after exposure to 100 ppm trichloroethylene were twice those seen after exposure to 50 ppm. However, the ratio increased to 2.5:1 towards the end of week. Similarly peak concentrations of trichloroethanol in the blood seen after intermittent exposure to 250 ppm trichloroethylene were equivalent to those following a continuous exposure to 50 ppm but again the ratio increased (to 1.25:1) towards the end of the week. Residual levels of trichloroethanol in the blood were always linearly related to exposure concentration. In volunteers exposed to 100 ppm, residual levels recorded immediately prior to exposure on days 2 and 5 rose from 1 to 1.6 μg/ml and residual levels following exposure to 50 and 250 ppm rose from 0.5 to 0.7-0.8 μg/ml. Following repeated exposure to 50 ppm, elimination from the blood was complete within 4 days of the last exposure. This indicates that some accumulation of trichloroethanol over the working week is likely.

The kinetics of trichloroethanol in the blood were reflected by the pattern of elimination of trichloroethanol in the urine. After a single exposure to 100 ppm trichloroethylene, it could be calculated from the graph that at least 70% of the trichloroethanol was eliminated in the first 24 hours post exposure. With repeated daily exposures to trichloroethylene, levels of trichloroethanol in the urine rose slightly between the 24-hour samples following exposure but dropped rapidly once exposure had stopped. Trichloroethanol was no longer detectable in urine within 5 days of the last exposure following repeated exposure to 50 ppm trichloroethylene.

In contrast to the kinetics observed for trichloroethanol, it was found that following a single exposure to 100 ppm trichloroethylene levels of trichloroacetic acid in plasma did not begin to fall immediately post exposure. Instead blood trichloroacetic acid levels continued to rise so that the peak levels of around 50 μg/ml were only attained 18-19 hours after cessation of exposure to trichloroethylene. Levels of trichloroacetic acid then began to decline very slowly and only 6% of the concentration in plasma had been eliminated over 36 hours. The half-life of trichloroacetic acid was calculated to be around 100 hours and this long half-life was attributed to extensive plasma binding of trichloroacetic acid (around 90% at this exposure level). This long half-life accounts for the substantial accumulation of trichloroacetic acid following repeated exposure to trichloroethylene. Plasma concentration data were only presented for repeated exposure to 50 ppm. After the first and second exposures, plasma concentrations continued to rise so that levels of trichloroacetic acid in the blood were higher immediately before exposure on days 2 and 3 respectively than at the end of the exposure on days 1 and 2 respectively. Thereafter levels did fall between exposures although the residual trichloroacetic acid concentration was always greater than the peak blood levels recorded 2 days previously. At the end of exposure on day 5, the peak concentration of trichloroacetic acid in the plasma was 3.5 times as great as the peak concentration on day 1 and there was no evidence that the system was approaching steady state towards the end of the week. Elimination of trichloroacetic acid from the blood was nearing completion 13 days after the last exposure. This shows that in humans the extensively plasma bound metabolite trichloroacetic acid is retained for a considerable length of time and there is therefore likely to be substantial accumulation of trichloroacetic acid over the working week. It is not clear how this accumulation will affect the extent to which this metabolite is bound to plasma proteins.

Urinary elimination of trichloroacetic acid was also slow. A relatively small amount of trichloroacetic acid was eliminated in the first 24 hours with only slight increases in the amount of trichloroacetic acid eliminated over the next 48 hours. With repeated exposure, the amount of trichloroacetic acid eliminated in urine increased with each day of exposure. Levels declined

gradually after the last exposure although trichloroacetic acid was still detectable in urine 12 days after the last exposure to 50 ppm trichloroethylene.

Monster et al. (1976, 1979) also followed the elimination kinetics of trichloroethanol and trichloroacetic acid from the blood of volunteers exposed either once or repeatedly to airborne trichloroethylene. Their results were consistent with those obtained by Ertle et al. (1972) and Müller et al. (1972, 1974) above: the half-life for trichloroethanol was 8-12 hours and that for trichloroacetic acid was 70-100 hours. Similar patterns of elimination were also reported by Nomiyama and Nomiyama (1971, 1977, 1979), Sato et al. (1977), Kostrzewski et al. (1993) and Vesterberg et al. (1976).

The urinary elimination kinetics of trichloroethanol, trichloroacetic acid and monochloroacetic acid have also been briefly examined in a study in which 5 volunteers were exposed for 5 hours to around 100 or 150 ppm (Soucek and Vlachova, 1960). It was reported that both trichloroethanol and trichloroacetic acid showed biphasic elimination patterns. For trichloroethanol the half lives for the two phases were calculated to be 24 and 40 hours and for trichloroacetic acid, the half-lives for the two phases were reported to be 50 and 70 hours respectively. The pattern of elimination of monochloroacetic acid most closely followed that of trichloroethanol and continued for between 48 and 168 hours. It was calculated that the half-life of this metabolite in urine was 15 hours. A second phase of elimination was observed for monochloroacetic acid. It is not clear why the half lives for trichloroethanol and trichloroacetic acid in urine are different from those determined from measurement of these two metabolites in the blood.

There have been reports of sex related differences in the toxicokinetics of trichloroethylene. Nomiyama and Nomiyama (1971, 1974a, 1974b) found that although men and women absorbed similar amounts of trichloroethylene (around 54-56% of the airborne concentration), women eliminated less as unchanged trichloroethylene than did men. Whereas women eliminated 16.7% of the airborne concentration as trichloroethylene, men eliminated 22.4%. These differences were reported to be statistically significant ($p<0.05$). Differences were also reported in the percentages of absorbed dose eliminated as the metabolites trichloroacetic acid and trichloroethanol. It was found that women eliminated around 44% of the absorbed dose as trichloroacetic acid and 43% of the absorbed dose as trichloroethanol, whereas men eliminated only 33% as trichloroacetic acid but 49% as trichloroethanol. The ratio of trichloroethanol to trichloroacetic acid was found to be significantly ($p<0.05$) greater in men than in women. However, it is not clear if these differences have any biological significance.

The metabolism of a number of substances is known to interact with the metabolism of trichloroethylene. Most important is alcohol, giving rise in some individuals to alcohol intolerance (trichloroethylene- or degreasers'-flush) the symptoms and signs of which are vasodilation, tachycardia, facial flushing, headache and hypotension which are similar to the side effects induced by chloral hydrate. In one study to investigate this phenomenon, a group of volunteers were exposed to trichloroethylene and then trichloroethylene with ethanol and levels of both parent compounds and metabolites determined in blood urine and exhaled air (Müller et al., 1975). In the first series of experiments, males aged between 20 and 26 who had been asked not to take any alcohol or other drugs prior to the experiment were exposed to 50 ppm trichloroethylene for 6 hours per day for 5 consecutive days. Two weeks later the exposure was repeated but subjects ingested alcohol both before and during exposure to give blood alcohol levels of 0.06%. A separate series of experiments were conducted in which volunteers received a single 6-hour exposure to 100 ppm trichloroethylene or trichloroethylene plus ethanol. In both studies, blood and urine samples were regularly taken for determination of trichloroethylene, trichloroethanol and trichloroacetic acid until elimination was nearly complete.

The results of the first series of experiments showed that simultaneous ingestion of ethanol caused marked reductions of around 40% in the levels of trichloroethylene metabolites in both blood and urine. It was found that the blood concentration of trichloroethylene was 2.5 times higher than in the absence of ethanol and the concentration of trichloroethylene in exhaled air was four times as great suggesting inhibition of metabolic clearance. No effect on the glucuronidation of trichloroethanol was shown. There was also a slight increase in ethanol and acetaldehyde levels in blood above levels expected without concomitant exposure to trichloroethylene. This issue was discussed by Davidson and Beliles (1991). In their review they quote other groups who have also studied alcohol intolerance (Sellers et al., 1972a, 1972b; Stewart et al., 1974; Nakanishi et al., 1978) and a consistent picture of competitive inhibition of metabolism leading to increased plasma levels of trichloroethylene, ethanol and acetaldehyde emerges. There is therefore clear evidence that simultaneous exposure to alcohol can have a significant effect on the toxicokinetics of trichloroethylene.

To summarise the inhalation toxicokinetics of trichloroethylene in humans, trichloroethylene is rapidly and extensively absorbed across the lungs reaching equilibrium with the blood after about 2 hours. It is distributed throughout the body, including the CNS and partitions preferentially into fat-rich tissues. It can cross the placenta in pregnant women. The elimination of trichloroethylene has three phases corresponding to richly perfused tissues ($t_{½}$ = 2-3 minutes), lean body mass ($t_{½}$ around 30 minutes) and fat-rich tissues ($t_{½}$ = 3.5-5 hours). The main route of elimination of trichloroethylene is via metabolism accounting for between 60 and 90% of the absorbed dose. The metabolites are eliminated mainly in the urine. The remaining trichloroethylene is eliminated unchanged in exhaled air.

Although metabolic pathways have not been characterised as extensively in humans as they have in animals, a number of common metabolites have been identified and it seems likely that the metabolic pathways are qualitatively the same. No evidence of saturation for any metabolic pathway has been found in humans although the exposure levels used were generally lower than those used in animal studies and thus saturable concentrations may not have been achieved. As with animals, trichloroethanol is the main urinary metabolite, although in humans a substantial proportion of the dose is also eliminated as trichloroacetic acid.

The kinetics of both trichloroethanol and trichloroacetic acid have been extensively studied. During exposure to trichloroethylene, levels of trichloroethanol in the blood rise rapidly but then drop rapidly once exposure has stopped. Most of the trichloroethanol arising from a single exposure to trichloroethylene is eliminated via the urine within the first 24 hours. With repeated exposure to trichloroethylene, some accumulation of trichloroethanol occurs so that over 5 days, peak levels in the blood were found to increase by 25-35%. At the dose levels used in this study, steady state was approached towards the end of the 5-day study. As with single trichloroethylene exposures, elimination of trichloroethanol is rapid once exposure to trichloroethylene stops. The half-life for trichloroethanol in human blood is around 10-12 hours which is considerably longer than the half-life of 2-5.3 hours determined for Sprague-Dawley rats and 0.5-2.7 hours determined for B6C3F1 mice.

In contrast, levels of trichloroacetic acid in the blood continue to rise for several hours after exposure to trichloroethylene has stopped and then decline very slowly. Repeated exposure to trichloroethylene leads to dramatic increases in blood trichloroacetic acid levels so that after 5 days exposure, levels in blood immediately post exposure after the 5th exposure are 3-fold higher than those found immediately after the first exposure. There are no signs of steady state being approached by the end of the week. This was attributable to the very tight plasma binding observed for trichloroacetic acid in humans. As would be expected, elimination via urine is prolonged. A half-life of 70-100 hours has been determined for trichloroacetic acid in the blood

which again is considerably longer than the half lives of trichloroacetic acid in Sprague-Dawley rats and B6C3F1 mice (5-7 and 4-7.7 hours respectively).

The kinetics of monochloroacetic acid have also been briefly examined. Urinary elimination followed a similar time course as that described for trichloroethanol. The half-life determined from urine data was 15 hours.

Although the effects of co-exposure to substances which share metabolic pathways with trichloroethylene have not been investigated as extensively in humans as they have in animals, there is evidence that co-exposure to alcohol can result in raised blood trichloroethylene levels. Competitive inhibition of metabolism of ethanol, resulting in elevated acetaldehyde levels has been suggested as the cause of alcohol intolerance sometimes seen in individuals occupationally exposed to trichloroethylene.

The operation of the quantitatively minor glutathione conjugation pathway in humans is discussed in detail in the carcinogenicity section (Section 4.1.2.8).

Oral

Although no toxicokinetics studies have been conducted in humans following exposure to trichloroethylene by the oral route, the case reports of individuals who accidentally or deliberately ingested trichloroethylene have shown that it is well absorbed orally and that routes of elimination correspond to those seen in humans exposed by the inhalation route. It is therefore likely that the findings of toxicokinetic studies conducted in humans exposed by the inhalation route are applicable to humans exposed by the oral route. This assumption is supported by strong evidence from animal studies that the toxicokinetics of trichloroethylene are similar by all relevant routes of exposure.

Dermal

Only two studies of dermal uptake of trichloroethylene in humans were available. In the most recent study, four volunteers each immersed one hand up to the wrist in trichloroethylene for 30 minutes and for the next 10 hours the concentration of trichloroethylene in breath and blood and the concentration of the metabolites trichloroacetic acid and trichloroethanol in urine were regularly measured (Sato and Nakajima, 1978). Steps were taken to prevent exposure to the vapour by any other route and all trichloroethylene was removed from the skin immediately after the 30-minute immersion.

In blood and breath, the maximum levels of trichloroethylene (around 2 μg/ml for blood and 0.28 μg/ml in breath) were found immediately post exposure. These levels were similar to those reported after a 4-hour inhalation exposure to 100 ppm trichloroethylene. Thereafter, trichloroethylene levels in blood and breath declined exponentially at a rate equivalent to that seen following inhalation exposure. The rate of uptake was not determined.

In an earlier study, three volunteers immersed only their thumbs in trichloroethylene for 30 minutes. The concentration of trichloroethylene in exhaled air was followed during exposure and for five hours afterwards (Stewart and Dodd, 1964). Again, steps were taken to ensure that no vapour was inhaled. A plot of concentration in breath versus time revealed that the peak concentration, around 0.5 ppm occurred within 15 minutes of the end of exposure. Two hours later, alveolar concentrations had declined to 0.08 ppm. The limits of detection for trichloroethylene in air were reported to be 0.01-0.005 ppm. The pattern of elimination was

similar to that described by Sato and Nakajima (1978) above. As in the previous study the rate of uptake was not determined.

The results of these two studies show that trichloroethylene is absorbed via the dermal route and that elimination kinetics are similar to those observed in experimental studies in humans using the inhalation route. There is no evidence to suggest that the distribution and metabolism are any different from that observed via inhalation.

4.1.2.1.3 Summary of toxicokinetics

Studies in experimental animals and humans have shown that trichloroethylene is rapidly and extensively absorbed by all routes of exposure. Once absorbed it readily distributes to all compartments within the body. Although trichloroethylene preferentially partitions into fat rich tissues, there is no evidence of prolonged retention at these sites. Trichloroethylene is predominantly cleared from the body by metabolism, accounting for 50 to 99% of the absorbed dose. Studies in humans and a variety of experimental animal species suggest that the metabolic pathways are common to all species. The major metabolic pathway in all species involves the initial conversion of trichloroethylene by cytochrome P450 to a transient epoxide. This epoxide undergoes intramolecular rearrangement to form trichloroacetaldehyde, which in turn is hydrolysed to form chloral hydrate. Chloral hydrate then acts as a substrate for alcohol dehydrogenase and chloral hydrate dehydrogenase to yield trichloroethanol and trichloroacetic acid respectively. A second quantitatively minor pathway involving conjugation with glutathione has also been identified in rats, mice and humans.

Although all species share common metabolic pathways, differences between species and strains have been identified in the saturability of trichloroethylene metabolism. This has been investigated most thoroughly in rats and mice. Whereas the metabolism of trichloroethylene shows little evidence of saturation in B6C3F1 and Swiss-Webster mice at oral doses of up to 2,000 mg/kg, there is clear evidence of saturation in all strains of rat following oral doses of 1,000 mg/kg. No evidence of saturation for any metabolic pathway has been found in humans although the exposure levels were generally lower than those used in animal studies and thus saturable concentrations may not have been achieved.

Routes of elimination for trichloroethylene are the same in humans and animals and there is no evidence that the route of exposure influences the route of elimination. Most unmetabolised trichloroethylene is exhaled; the percentage which is exhaled increases when metabolism becomes saturated. Other metabolites which have been detected in exhaled air include carbon dioxide, carbon monoxide and a small amount of trichloroethanol. Metabolites of trichloroethylene are predominantly eliminated in the urine with a small proportion eliminated in the bile and faeces. Other routes of elimination have not been investigated.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

The acute toxicity of trichloroethylene has been extensively investigated in animals. It has low toxicity by the inhalation and oral routes and very low toxicity following percutaneous administration.

For more detailed investigation of several specific organ toxicities, studies have been conducted using the intraperitoneal route of administration: relevant results from these are briefly referred to at the most appropriate points in the text, but toxicity following intraperitoneal administration is not addressed *per se*.

Inhalation

General toxicity

The inhalation LC_{50} value for trichloroethylene in the rat has been reported to be about 26,000 ppm for a one-hour exposure (Vernot et al., 1977) and 12,000 ppm (65 mg/l) after a four-hour exposure (Siegel et al., 1971). A six-hour LC_{50} value of 5,918 ppm was obtained in a study in male Sprague Dawley rats (Bonnet et al., 1980). Few further details were reported except that there were no macroscopic changes observed in the lung, liver and kidney. The lowest lethal dose was reported as 4,800 ppm (26 mg/l) following a four-hour exposure (Adams et al., 1951). In various studies, the main signs of toxicity were stupor, irritation of the eyes and respiratory tract, poor co-ordination, CNS depression and respiratory failure (Adams et al., 1951; Utesch et al., 1981; Schumacher and Grandjean, 1960). Full anaesthesia was seen following exposure for four hours at trichloroethylene concentrations of about 5,000 ppm (27 mg/l) and above (Adams et al., 1951). Anaesthesia occurred within ten minutes at 15,000 ppm.

In the mouse, a four-hour LC_{50} value of 8,450 ppm (46 mg/l) has been determined for trichloroethylene and in a briefly reported study, a 6-hour LC_{50} value of 5,857 ppm was obtained for 99% pure trichloroethylene in female (OF1 strain) SPF mice (Friberg et al., 1953; Gradiski et al., 1978). No further information on signs of toxicity was reported. Complete anaesthesia occurred after about five minutes exposure of mice to 12,000 ppm and about ten minutes exposure to 7,000 ppm of' trichloroethylene (Friberg et al., 1953). In another study, about 50% of the animals were anaesthetised after 46 minutes exposure to trichloroethylene at 5,500 ppm (Gehring, 1968).

The effects of single inhalation exposures of rabbits to trichloroethylene were reported in an old and poorly-reported study. Rabbits survived exposure to about 15,000 ppm of trichloroethylene for 40 minutes but some deaths were recorded if the duration of exposure was extended (Pennarola et al., 1966). Signs of toxicity included hyperactivity, dyspnoea and narcosis. At autopsy (time relative to cessation of exposure not given), gross and/or microscopic congestion and/or oedema were observed in the liver, lung, heart, kidney, spleen and brain; no changes were observed in the adrenals. In another study, exposure of rabbits to 9,250 ppm of trichloroethylene for one hour was reported to induce anaesthesia (Truhaut et al., 1972).

Exposure of dogs to 30,000 ppm of trichloroethylene rapidly caused salivation and uncontrolled limb movements followed by loss of consciousness, convulsions and death within about 20 minutes (Baker, 1958). Post mortem examinations were limited to the nervous system and no microscopic changes were observed. In another study, anaesthetised dogs (number of animals not given) were exposed to 500 to 50,000 ppm trichloroethylene for ten minutes via tracheal cannulae in a study of respiratory, bronchopulmonary and cardiovascular effects (Aviado et al., 1976). The animals used in this study were severely compromised by surgical procedures. No significant effects were recorded on pulmonary mechanics, but there were dose-related depressive effects on myocardial contractility, with decreased cardiac output and hypotension occurring at the higher exposure levels.

In summary, 4-hour LC_{50} values of 12,000 ppm (65 mg/l) and 8,450 ppm (46 mg/l) have been reported for rat and mouse, respectively. The main signs of toxicity observed were those typical of CNS depression.

Pulmonary toxicity

A number of single-exposure studies have been conducted in rats and mice. The observations made in these studies are of particular significance with regard to the mouse lung carcinogenicity findings discussed below.

No treatment-related effects were seen in the lungs of female Alpk:APfSD rats sacrificed 24 hours after a 6-hour inhalation exposure to 0, 500 or 1,000 ppm (0, 2.7 or 5.4 mg/l) of Aristar grade trichloroethylene (Odum et al., 1992). In an unseen study cited by Odum et al., "some flattening of the bronchiolar epithelium" was reported to occur following inhalation exposure of rats to high concentrations (eg 8,000 ppm) of trichloroethylene (Kurasawa, 1988).

Pulmonary effects following single inhalation exposures of mice to trichloroethylene have been investigated in detail. In a well-reported study, female CD-1 mice were exposed to 20, 100, 200, 450, 1,000 or 2,000 ppm trichloroethylene (Aristar grade) for 6 hours (Odum et al., 1992). Further groups of mice were exposed to either 100 ppm anhydrous chloral (Analar grade) for 6 hours or 100 or 500 ppm trichloroethanol for 6 or 2 hours respectively. Control groups, exposed to air alone, were included in each experiment. All animals were sacrificed 24 hours after the start of exposure.

The only clinical sign of toxicity reported for the mice exposed to trichloroethylene was mild anaesthesia at higher doses. No effects on lung/bodyweight ratios were seen, but dose-dependent vacuolation of Clara cells was observed in all mice exposed to trichloroethylene, the number of cells affected increasing with dose. A small number of Clara cells were affected at 20 ppm and most were affected at 200 ppm (approximately 1 mg/l). At higher dose levels the vacuolation of the Clara cells was accompanied by pyknosis of bronchiolar epithelium and at 2,000 ppm focal loss of bronchiolar epithelium was evident with exudate present in the lumen. No evidence of toxicity was seen in other cell types.

In the mice exposed to chloral or trichloroethanol, deep anaesthesia was reported. Lungs from chloral-treated mice showed similar damage to those treated with trichloroethylene but the effects were more severe and were accompanied by some alveolar necrosis and epithelial desquamation. In contrast, minimal toxicity was seen in trichloroethanol-treated mice: 66% and 33% of animals exposed to 100 or 500 ppm, respectively, were unaffected by this metabolite and only mild Clara cell lesions, without vacuolation, were seen in the remaining animals. These results suggest that chloral may be the metabolite responsible for pulmonary toxicity in the mouse. Biochemical evidence was obtained, using preparations of mouse lung Clara cells, that the cell specificity of the lesion is due to the particular metabolic pattern of trichloroethylene in Clara cells. Their ability to metabolise chloral to trichloroethanol and to conjugate trichloroethanol is low compared with mouse hepatocytes though both cell types are equally able to metabolise trichloroethylene to chloral. As mentioned above, no effects were seen in the lungs of female rats exposed to trichloroethylene in the same study.

In another part of this study, cytochrome P-450 activities in sonicates of Clara cells from the lungs of trichloroethylene-exposed mice were shown to be reduced in a dose-dependent manner: there was virtually no effect at 20 ppm but at 200 ppm the activities of certain cytochrome P-450 enzymes were shown to be 60 to 75% of control values (Odum et al., 1992). Cytochrome P-450 activities were also reduced in microsomes isolated from the whole lung of rats exposed for six

hours to 500 or 1,000 ppm of trichloroethylene. These effects were considered to be partly a reflection of general cell toxicity and partly a consequence of suicide inhibition/cytochrome P-450 degradation.

Similar cell-specific toxicity has also been seen in the lungs of male mice. Male B6C3F1 mice were exposed for 30 minutes to 500, 1,000, 2,000, 3,500 or 7,000 ppm trichloroethylene and sacrificed after 2 and 24 hours then 2, 5 and 7 days (Villaschi et al., 1991). A control group of mice was sacrificed 48 hours after exposure to air under the same conditions. The lungs of the animals were fixed in situ and taken for histopathological and ultrastructural examinations.

Light microscopy showed cell damage, consisting of varying degrees of cytoplasmic vacuolation, which was confined to non-ciliated cells with the morphological characteristics of Clara cells. The damage was significantly greater in bronchi with diameters $\leq$ 0.4 mm than in larger bronchi and was maximal at two days after exposure. There was a clear positive correlation with dose at two days only (e.g. for bronchi with diameters of $\leq$ 0.4 mm, the mean percentage of vacuolated cells was 0.2 in control animals, 1.8 at 500 ppm of trichloroethylene, 3.0 at 1,000 ppm, 8.8 at 2,000 ppm, 13.1 at 3,500 ppm and 23.4 at 7,000 ppm; $p < 0.01$). The authors reported this damage to be maximal at 24 hours but, in the absence of data for control animals at this time point, the very high, non-dose-related percentages of vacuolated non-ciliated cells (average values of 67 $\pm$ 8.5% in bronchi of $\leq$ 0.4 mm and 34 $\pm$ 13% in larger bronchi) cannot be interpreted specifically in relation to trichloroethylene exposure. However, in the study summarised above (Odum et al., 1992), "most" mouse lung Clara cells were seen to be vacuolated 18 hours after a six-hour exposure of mice to 200 ppm of trichloroethylene.

By seven days after exposure, the proportion of vacuolated non-ciliated cells in trichloroethylene-exposed animals had returned to control levels. Electron microscopy revealed damage consisting of dilatation of the endoplasmic reticulum in non-ciliated cells only. This was seen at all dose levels. Alveolar type II cells (which are also metabolically competent) were unaffected by trichloroethylene in this study.

Further groups of similarly exposed mice were additionally given 1 μCi/g bodyweight of ^{3}H-thymidine intraperitoneally 1 hour before sacrifice (at 48 hours, 5 days and 7 days after exposure; controls at 48 hours only) in order to study cell turnover. The percentage of ^{3}H-thymidine labelled cells (indicating cell replication) increased to a maximum of 13.5 $\pm$ 6.8 % at 48 hours post-exposure and then decreased to the level seen in controls (virtually zero) by seven days after exposure. Dividing cells were observed to have the morphological characteristics of Clara cells. From the results of the histopathological and labelling studies it is apparent that mouse lung Clara cell damage reaches a peak within two days of exposure to a single, 30-minute, exposure to trichloroethylene at concentrations of 500 ppm and above. Restoration of the normal proportion of non-ciliated cells occurs both via recovery of less damaged cells and replication of residual non-ciliated cells.

The specific toxicity of trichloroethylene towards certain metabolically competent cells in mouse lung has been substantiated in a series of studies in which single high doses (2,000 to 3,000 mg/kg body weight) of unlabelled or ^{14}C-labelled trichloroethylene were given intraperitoneally (Forkert et al., 1985; Forkert and Troughton, 1987; Forkert and Birch, 1989). Covalent binding of trichloroethylene and/or its metabolites was shown to occur within one hour of dosing and perturbations of microsomal parameters paralleled the morphological changes in non-ciliated (Clara) cells.

Thus, it can be concluded that, in mice but not in rats, single exposures to trichloroethylene (at concentrations of 20 ppm and above for 6 hours, 500 ppm and above for 30 minutes) cause

damage to a specific cell type in the lung (the Clara cell). The amount of damage reaches a peak 24 to 48 hours after exposure. Recovery is complete by seven days after exposure. Biochemical studies have shown that these changes may be related to the accumulation of chloral (an intermediate metabolite of trichloroethylene) in mouse lung Clara cells as a consequence of the relative inability of these cells (compared with mouse hepatocytes) to metabolise chloral any further. The lowest observable effect level for mouse lung toxicity was 20 ppm of trichloroethylene for 6 hours, but no pulmonary toxicity was observed in rats exposed to 1,000 ppm for the same period of time. The mode of action for pulmonary toxicity has been further investigated in relation to carcinogenicity (see Section 4.1.2.8). These studies indicated that the pulmonary toxicity seen in mice was unlikely to be of relevance to humans.

Behavioural and central nervous system effects

Volatile solvents such as trichloroethylene can be expected to have CNS-depressant effects at high atmospheric concentrations. At concentrations below those causing respiratory failure, these effects are usually transient. This property has, for trichloroethylene, been utilised in the use of the substance as an anaesthetic. Hence, the effects reported in studies in which high (near anaesthetic) concentrations of trichloroethylene were used are predictably those typical of CNS depression. However, data from studies in animals on behavioural and central nervous system effects, following exposure to sub-anaesthetic concentrations of a substance, can be particularly useful as human data on this type of effect are often subjective.

The effects of trichloroethylene on the behavioural activity of rodents following single inhalation exposures have been investigated in a number of studies conducted in the 1960s and 1970s (Grandjean, 1960 and 1963; Grandjean and Battig, 1964; Wolff, 1976). Exposure levels ranged from 120 to 1,600 ppm and exposure times from one hour to eight hours. Fatigue, as measured by swimming times and spontaneous climbing activity, was increased in rats exposed to trichloroethylene for 6 hours at a concentration of 800 ppm, but not at 400 ppm, while motor activity and, in a T-maze, spontaneous alternation were reduced. In mice, spontaneous activity was reduced by 50% following a 2-hour exposure to around 2,000 ppm of trichloroethylene. Overall, the “no effect level” in these studies is a six-hour exposure to 400 ppm of trichloroethylene.

In a recent but not clearly presented study, the blood concentrations of trichloroethylene associated with specific behavioural changes have been studied in male Wistar rats (Kishi et al., 1993). A group of 8 animals trained to press a lever in response to a light stimulus to avoid receiving an electric shock were exposed (whole body) sequentially to 0, 250, 500, 1,000, 2,000 and 4,000 ppm of trichloroethylene (purity not stated) in ascending order with 10- to 20-day intervals between exposures. In each case, exposure lasted for 4 hours and occurred during the dark cycle of the 12-hour light/dark schedule, a time when rats would normally be active.

Concentration-related decrements in shock avoidance performance were noted at all exposure levels. Exposure to 250 ppm affected performance within 140 minutes. Similar effects were observed following 80 minutes of exposure at 500 ppm, and at 1,000 and 2,000 ppm changes were observed within 20 minutes of starting exposure. There was also an increase in the time between receiving the light stimulus and pressing the lever at the higher exposure levels. At 4,000 ppm, performance decreased markedly and anaesthesia was noted: all rats became ataxic and failed to respond to both the light stimulus and the electric shock. One rat from this group died during the post-exposure observation period but the remaining seven showed gradual recovery of performance. Performance disorders were still being recorded at 140 minutes post-exposure and, in all except the highest dose group, they were often more marked after cessation of exposure.

The results of the blood analyses indicated that blood concentrations of 10 to 40 μg/ml (levels found at the end of a 4-hour exposure to 250 and 500 ppm) corresponded to slight performance decrements and some signs of CNS depression. Higher blood levels (100 μg/ml or more) achieved after a 4-hour exposure to 2,000 or 4,000 ppm (10.8 or 21.6 mg/l), were associated with CNS depression and anaesthesia.

The effects of single exposures to high, but sub-anaesthetic, concentrations of analytical grade trichloroethylene on the central vestibular system have been studied by recording stimulated eye movements in a well-conducted experiment in rats (Niklasson et al., 1993). Eighteen female and 10 male pigmented rats were exposed to 2,700, 4,200, 6,100 and 7,200 ppm of trichloroethylene for one hour. These exposure levels had been observed to cause some behavioural changes (loss of righting reflex and depression of avoidance reactions to nose or tail touch) in preliminary tests. The rats in the main study were initially tested pre-exposure to obtain individual baseline readings. Provocation and recording of saccades (quick reposition of the eyes) began ten minutes into the exposure and, thereafter, the mean slow-phase eye velocities (SPVs) of nystagmus (rapid, involuntary movement of the eyeball) were recorded during vestibular and/or optokinetic stimulation.

Changes in the measured parameters were seen at all the trichloroethylene exposure levels. Concentration-related reductions in ability to make compensatory eye movements following optokinetic stimulation and in maximal SPVs following vestibular stimulation, and a concentration-related prolongation of nystagmus were recorded. When optokinetic and vestibular stimulation were applied simultaneously, no nystagmus occurred in control animals indicating suppression of vestibular reaction to motion by visual input. Exposure to trichloroethylene caused concentration-related inability to suppress the vestibular influence. Changes in the generation of saccadic eye movements were also seen in exposed animals.

Compensatory eye movements are elicited through the vestibular-oculomotor reflex, which requires an optokinetic support to function properly. Interpretation of the patterns of response seen in this study indicated that trichloroethylene affects a part of the reflex system: the cerebro-vestibular circuit. It is postulated by the authors, and by others cited, that substance-induced changes of the lipid membrane of neuronal cells causing changes in membrane fluidity may precede changes in receptor protein functions with consequent effects on neurotransmission. However, these subtle changes recorded during exposure to trichloroethylene concentrations at which CNS depression would be expected, are considered to be of little biological significance.

In a study conducted in mice, general motor activity was used as a marker for behavioural effects (Kjellstrand et al., 1985). NMRI mice were exposed to trichloroethylene for one hour during the night (the normal active period) and motor activity was monitored during and after cessation of exposure. The trichloroethylene was commercial trichloroethylene containing “the usual stabilisers”. Fifty-four mice were exposed to trichloroethylene at 380 or 480 ppm, 27 to 570, 700, 900 or 1,200 ppm and 14 to 1,100, 1,800, 2,300 or 3,600 ppm. At the highest trichloroethylene levels, motor activity initially (within five minutes) increased but at 900 ppm there was little or no effect. At 700 ppm, slightly decreased motor activity was recorded and this effect was gradually lost at the lower exposure levels. On termination of exposure there was a rapid change in motor activity and animals in which an initial increase in motor activity had been observed rapidly became hypoactive.

The rate of change in trichloroethylene concentration in the atmosphere seemed to be the critical factor affecting behaviour rather than either the final concentration achieved or any clear relationship between a particular actual atmospheric concentration and an effect. The possibility of non-specific effects in response to the odours (though not the possible irritancy) of the

solvents used in this study was ruled out as no effects on motor activity were induced during exposure to a strongly scented substance (presumably at a very low concentration). A similar direct action of trichloroethylene on the membranes of the cells of the central nervous system to that outlined for the study above is proposed and seems plausible.

Summary of behavioural and central nervous system effects

In summary, in studies designed to investigate the effects of trichloroethylene on behaviour and the central nervous system, increased fatigue was observed in rats exposed to 800 ppm or more of trichloroethylene for 6 hours, but there were no effects on behavioural parameters when the exposure level was 400 ppm or less. In mice, general motor activity was affected by exposure to trichloroethylene for 1 or 2 hours at 1,000 ppm and above but there was little effect at 700 ppm and below. In a poorly reported study, slight effects on shock avoidance performance accompanied by some signs of CNS depression were reportedly observed in rats exposed to 250 ppm (1.35 mg/l) or more of trichloroethylene for 4 hours, following which blood concentrations of trichloroethylene reached 10 to 40 μg/ml. Blood trichloroethylene concentrations of 100 μg/ml or more (as seen following exposure to 2,000 to 4,000 ppm of trichloroethylene for 4 hours) were associated with CNS depression and anaesthesia. Subtle effects on stimulated eye movements were observed in rats exposed to high atmospheric concentrations (> 2,700 ppm) of trichloroethylene for 1 hour, but these observations are considered to be of little toxicological significance. Thus, there were no significant effects on behavioural parameters or the CNS in rodents exposed to trichloroethylene at concentrations of (or equivalent to) 400 ppm for 6 hours.

Hepatotoxicity

Significant ($p < 0.05$ to $p < 0.001$) increases in serum levels of enzyme markers of liver dysfunction (alanine aminotransferase, ALT, and/or aspartate aminotransferase, AST) were noted in rats exposed to about 10,000 ppm of trichloroethylene for one or two hours and killed 16 to 48 hours post-exposure (Carlson, 1974; Cornish and Adefuin, 1966). Serum ALT levels were doubled after exposure of rats to 2,000 ppm of trichloroethylene for four hours (Cornish and Adefuin, 1966). At necropsy, slight effects on the liver only were noted, including increased weight, oedema and fatty infiltration (Adams et al., 1951; Carlson, 1974; Cornish and Adefuin, 1966). Transient increases in AST levels were also reportedly seen in the serum of rats exposed to lower concentrations of trichloroethylene (100 to 1,000 ppm) for six hours (Deguchi, 1972). The AST levels were elevated at 24 and 48 hours post-exposure but normal at 72 hours; there were no changes in serum ALT levels in this study. Pre-treatment of rats with alcohol, phenobarbitone or other enzyme inducers, enhanced the toxicity of trichloroethylene and increased the extent of the effects observed in the liver (Carlson, 1974; Cornish and Adefuin, 1966; Molsen et al., 1977a; Molsen et al., 1977b).

In other early studies, biochemical indications of toxicity in the liver of the mouse were seen following single inhalation exposures to trichloroethylene concentrations varying from 800 ppm for three hours to 18,000 ppm for one hour (Gehring, 1968; von Heim et al., 1966; Ogata et al., 1968).

In a number of studies, intraperitoneal administration of single doses (250 to 3,000 mg/kg body weight) of trichloroethylene, undiluted or in one of a variety of vehicles, also showed that dose-related adverse effects on the liver or on biochemical indicators of such effects were induced in rats and mice at dose levels of 1,500 mg/kg body weight and above (Allemand et al., 1978; Cornish et al., 1973; Elcombe et al., 1981; Klaassen and Plaa, 1966; Pessayre et al., 1979; Rigaud et al., 1977).

In summary, single inhalation exposures of rodents to trichloroethylene at concentrations equivalent to or more than 800 ppm for three hours is associated with transient increases in serum levels of enzyme markers of liver dysfunction. These effects were increased further in animals pre-treated with alcohol or other enzyme inducers.

Nephrotoxicity

The effects of trichloroethylene on the kidney have been specifically investigated using groups of 6 male F344 rats exposed (whole body) to 0, 1,000 or 2,000 ppm (0, 5.4 or 10.8 mg/l) of trichloroethylene for 6 hours (Chakrabarti and Tuchweber, 1988). Rats were housed in metabolism cages for 24 hours post-exposure for collection of urine. At the end of this period, blood was taken and kidneys collected for determination of p-aminohippurate accumulation in cortical slices (a measure of proximal tubule function).

The results showed urinary glucose, proteins, γ-glutamyltranspeptidase (a brush border enzyme) and serum urea nitrogen were significantly elevated ($p < 0.05$) at both doses compared to control rats (with a clear dose relationship only for urinary proteins), and urinary N-acetyl-β-glucose-D-aminidase levels were significantly elevated ($p < 0.05$) at 2,000 ppm, suggesting an impairment of both tubular and glomerular functions. Further evidence of impaired tubular function was given by the reduced capacity for accumulation of p-aminohippurate in renal cortical slices observed at both exposure levels. There were no histopathological examinations made of the kidneys and no comments about their macroscopic appearance. Given that no evidence of kidney toxicity was apparent from earlier studies in rodents using much higher exposure levels of trichloroethylene, the significance of these observed changes is unclear. It is possible that they are transient and no effects would have been seen if the animals had been sacrificed later. However, in the earlier studies in rats, biochemical indicators of kidney toxicity may not have been examined in such detail.

Parallel studies were conducted by the same group using the intraperitoneal route of exposure in male F344 rats pre-treated with phenobarbitone and killed 24 hours after dosing with trichloroethylene (Chakrabarti and Tuchweber, 1988). Nephrotoxicity was assessed using biochemical methods, and urinary excretion of trichloroethylene metabolites was measured. Animals treated with the highest dose level only (3,000 mg/kg bodyweight) of trichloroethylene showed evidence of both saturation of metabolism and nephrotoxicity. Thus, it can be postulated that the acute, possibly transient, nephrotoxicity of trichloroethylene is expressed only when the capacity to metabolise trichloroethylene is exceeded. As described in Section 4.2.1, above, saturation of the metabolic pathways for trichloroethylene occurs in rats at dose levels (oral) of 1,000 mg/kg body weight and above.

There was no information on mouse kidney effects following single inhalation exposures to trichloroethylene. Intraperitoneal administration of trichloroethylene in corn oil caused a significant decrease ($p < 0.05$) in p-aminohippurate accumulation in renal cortical slices taken 24 hours post-exposure from male ICR mice given 700 to 1,900 mg/kg body weight, but no effects on kidney function were seen in male Swiss-Webster mice given 2,900 mg/kg (Klaassen and Plaa, 1966; Kluwe et al., 1978; Kluwe et al., 1979).

In summary, there is limited evidence, from clinical chemistry, urinalysis and *in vitro* investigations in one study, that single inhalation exposures of rats to trichloroethylene (1,000 and 2,000 ppm for 6 hours) may cause kidney toxicity.

Cardiac sensitisation

Trichloroethylene, at high concentrations, has been shown to sensitise the heart to the action of catecholamines. In rats pre-treated with phenobarbitone or Aroclor 1254, exposed for one hour to 0 or 25,000 ppm of trichloroethylene and given up to 4 μg/kg body weight of adrenalin at various times during the exposure period, cardiac arrhythmias were recorded in the animals exposed to trichloroethylene (White and Carlson, 1979). There was an exposure-time-related increase in cardiac sensitivity to adrenalin.

A similar set of experiments conducted in rabbits was inconclusive in this study but in later studies by the same group, rabbits (in groups of 3 or 6 animals) which had not been pre-treated displayed adrenalin-induced arrhythmias (specifically, premature ventricular contraction) during exposure for 1 hour to trichloroethylene at concentrations of 6,000 and 8,000 ppm, but not at 2,000 ppm. Exposure to increasing concentrations of trichloroethylene resulted in an increased incidence of arrhythmias after shorter exposure times and following stimulation with lower levels of adrenalin (White and Carlson, 1981a). Administration of chloral hydrate (50 mg/kg body weight, given intravenously) to a group of four rabbits gave blood levels of trichloroethanol and trichloroacetic acid very much higher (15 and 50 times, respectively) than those achieved during exposure to 8,000 ppm of trichloroethylene when measured at the same time after the start of exposure (White and Carlson, 1981a). These relatively high blood levels were maintained for the duration of the experiment. No arrhythmias were induced by administration of adrenalin to the chloral-treated rabbits. This suggests that it is trichloroethylene itself which sensitises the heart to adrenalin. Rabbits pre-treated with ethanol (1,000 mg/kg body weight) 30 minutes before exposure to trichloroethylene (6,000 ppm for 1 hour) developed adrenalin-induced arrhythmias sooner and at lower doses of adrenalin than those not also given ethanol (White and Carlson, 1981b).

Cardiac arrhythmias were noted in 7/12 dogs exposed to 10,000 ppm of trichloroethylene for ten minutes and given intravenous adrenaline (8 μg/kg body weight); similar effects were noted in 1/12 dogs exposed to 5,000 ppm (Reinhardt et al., 1973).

Hence, it has been shown that single exposures of rats (pre-treated with enzyme inducers) and rabbits to trichloroethylene at atmospheric concentrations of $\geq$ 6,000 ppm for one hour can cause cardiac hyperreactivity to catecholamine stimulation. Cardiac sensitisation was not seen in rabbits exposed to 2,000 ppm of trichloroethylene for one hour. The observed effects were potentiated in rabbits pre-treated with ethanol. Similar effects also occurred in dogs exposed for short periods to trichloroethylene at concentrations $\geq$ 5,000 ppm.

Immunotoxicity

Aranyi et al. (1986) investigated the effects of single 3-hour inhalation exposures in mice on their susceptibility to experimentally induced streptococcus aerosol infection and pulmonary bactericidal activity against inhaled *Klebsiella pneumoniae*. The airborne exposure concentrations were 2.5, 5, 10, 25 or 50 ppm. There were approximately 30 mice in each group, and 5 or 10 replicate challenges for the streptococcus study and between 17 and 24 mice in each group for the bactericidal assay. At 25 and 50 ppm, mortality from streptococcal pneumonia was considerably higher than for the control groups; at lower concentrations mortality in the treated groups was similar to controls. Bactericidal activity was reduced at 50, 25 and 10 ppm, but the response was not concentration dependent and consequently cannot be considered to be treatment related. The toxicological significance of these findings is unclear because these assays have not been validation as toxicity tests for chemicals.

Oral

Oral LD_{50} values for trichloroethylene have been reported in the range 5,400 to 7,200 mg/kg body weight in rats and about 2,900 mg/kg in mice, the substance being given undiluted, in water or in vegetable oil (Aviado et al., 1976; Domenico et al., 1977; Jones et al., 1958; Kinkead and Wolfe, 1980; Smyth et al., 1962; Smyth et al., 1969). The lowest lethal dose for trichloroethylene in corn oil was found to be 5,600 mg/kg body weight in rats and 10,000 mg/kg in mice (National Cancer Institute, 1976). CNS depression was noted in mice following single oral doses of 700 mg/kg body weight and above of trichloroethylene in olive oil; liver lesions (fatty infiltration) were noted at necropsy (Jones et al., 1958). Alterations in serum biochemistry, including transiently increased AST and ALT activities, were shown in rabbits given 1,700 mg/kg body weight of trichloroethylene, indicating effects on liver function (Fujii, 1975). All dogs survived single oral doses of 3,000 to 6,000 mg/kg body weight of trichloroethylene given as a suspension in acacia mucilage (Barsoum and Sadd, 1934).

Dermal

The LD_{50} value of trichloroethylene in rabbits when administered by the dermal route, using an occlusive dressing, has been reported as being greater than 29,000 mg/kg body weight (Smyth et al., 1962; Smyth et al., 1969). In another study, the LD_{50} value in the rabbit following a 24-hour exposure with a semi-occlusive dressing was reported to be > 20,000 mg/kg (Kinkead and Wolfe, 1980). No further details were given. No deaths occurred in guinea-pigs following dermal application of 7,800 mg/kg body weight of trichloroethylene using a similar method: the only sign of toxicity noted was reduced weight gain during the 35-day post-exposure observation period (Wahlberg and Boman, 1979).

Other

A number of useful confirmatory/mechanistic studies on the toxicity of trichloroethylene have been conducted using the intraperitoneal route. These have been briefly mentioned above in relation to liver and lung toxicity in mice and nephrotoxicity in rats. Other studies have also been conducted in several species using various routes of exposure, and there are some *in vitro* studies available. These studies sometimes form the whole subject of a report or they may be elements of a series of experiments within a report. They have all been assessed, but none was considered to provide any additional information useful for hazard identification or human health risk assessment (eg Barsoum and Saad, 1934; Klaunig et al., 1989; McCarty et al., 1992; Odum et al., 1992; Plaa et al., 1958; Tomenius et al., 1979; Wirtschafter and Cronyn, 1964).

Summary of acute toxicity in animals

Trichloroethylene has low acute toxicity by the inhalation route of exposure; 4-hour LC_{50} values of 12,000 ppm (65 mg/l) and 8,450 ppm (46 mg/l) were obtained for rats and mice, respectively. The main signs of toxicity were those typical of CNS depression. Full anaesthesia was induced following a 4-hour exposure to 5,000 ppm (27 mg/l) of trichloroethylene. Blood concentrations of trichloroethylene of 100 μg/ml or more, achieved in one study following 4-hour exposures to 2,000 and 4,000 ppm of the substance, were associated with CNS depression and anaesthesia.

Pulmonary toxicity was observed in mice, but not in rats, following single inhalation exposures to trichloroethylene at concentrations of 20 ppm and above for 6 hours or 500 ppm and above for 30 minutes. This toxicity was essentially confined to a single cell type (the Clara cell). Cell damage reached a peak 24 to 48 hours after exposure and recovery was complete by 7 days post-exposure. Mouse lung Clara cell toxicity appears to be related to the accumulation of chloral (an

intermediate metabolite of trichloroethylene) in the cells. The lowest observable effect level for mouse lung toxicity was 20 ppm of trichloroethylene for 6 hours, but no pulmonary toxicity was observed in rats exposed to 1,000 ppm for the same period of time.

There were no significant effects on CNS or behavioural parameters in rodents exposed to trichloroethylene at $\leq$ 500 ppm (2.7 mg/l) for 4 hours or 400 ppm for 6 hours.

Transient increases in serum enzyme markers of liver dysfunction were observed following exposure of rats and mice to trichloroethylene at concentrations of around 1,000 ppm for 2 to 6 hours, and increased liver weight with fatty infiltration has been reported. These effects were enhanced if the animals were pre-treated with alcohol.

Biochemical indications of nephrotoxicity have been reported in only one study in rats exposed to trichloroethylene at $\geq$ 1,000 ppm for 6 hours.

Cardiac hyperreactivity to catecholamine stimulation was observed in rabbits exposed to trichloroethylene at $\geq$ 6,000 ppm, but not at 2,000 ppm, for one hour. The effects were potentiated in animals pre-treated with alcohol. Cardiac sensitisation was also observed in rats and dogs exposed to very high concentrations of trichloroethylene (25,000 ppm for 1 hour and 10,000 ppm for 10 minutes, respectively).

Overall, the NOAEL for single inhalation exposures of animals to trichloroethylene are 400 ppm for 6 hours, 500 ppm for 4 hours, although subtle transient effects on mouse lung Clara cells were seen at 20 ppm for 6 hours; there was evidence to suggest that these effects have a high degree of species specificity. In general, mice were more sensitive to the toxic effects of single inhalation exposures to trichloroethylene than rats, and in all species in which alcohol pre-treatment was used, the effects of trichloroethylene were enhanced. Mode of action studies has indicated that the pulmonary toxicity seen in mice is unlikely to be of relevance to humans.

Following single oral doses of trichloroethylene, LD_{50} values in the range 5,400 to 7,200 mg/kg body weight were reported for rats. A lower value, 2,900 mg/kg, was obtained for mice. CNS depression and effects on the liver (fatty infiltration, transient increases in serum enzyme markers of hepatic dysfunction) were the only signs of toxicity reported.

No dermal LD_{50} values are available. In rabbits and guinea pigs, LD_{50} values exceed 20,000 mg/kg body weight and 7,800 mg/kg, respectively.

4.1.2.2.2 Studies in humans

Inhalation

Trichloroethylene has been used as an anaesthetic and has also been used by solvent abusers. Data are available from both of these exposure scenarios and also from accident reports and studies in volunteers. Some accident reports and all the reports of solvent abuse are particularly poorly presented and/or cannot be interpreted clearly with regard specifically to trichloroethylene toxicity. Data from such reports have therefore not been included in this review.

Neurological damage, particularly involving the trigeminal and optic nerves, has occasionally been reported, especially in earlier reports, following accidental exposure of people to narcotic concentrations of trichloroethylene. There is some evidence to suggest that this effect was caused by decomposition products of trichloroethylene, particularly dichloroacetylene, rather than by the substance itself. Trichloroethylene may decompose under the influence of heat and light or in

contact with strong alkalis, to form more toxic products such as phosgene and dichloroacetylene (Mertens, 1993; Saunders, 1967; Sax and Lewis, 1989). Trichloroethylene usually contains stabilisers to protect against such decomposition. Neurotoxicity has been noted after use of trichloroethylene as an anaesthetic, but only when closed-circuit systems containing soda-lime were used, introducing the possibility of alkaline decomposition to dichloroacetylene. Evidence has been cited supporting the claim that no cases of trigeminal nerve toxicity have been associated with exposure to purified or stabilised trichloroethylene under conditions where decomposition was unlikely (Grant, 1974). The results reported for some of the studies summarised below therefore need to be carefully interpreted in relation to the possible specific causative agent.

Accident reports

Indications of liver toxicity were observed in a well-presented report on three male workers hospitalised after an acute exposure to trichloroethylene at work (Kostrzewski et al., 1993). The three men had no known previous exposure to trichloroethylene. They entered a tank containing an unknown quantity of the substance. Loss of consciousness occurred within five minutes of entering the tank, but the men remained in the tank for 20, 25 or 30 minutes in total. They were admitted to hospital four hours after removal from the tank. Two of the men fully recovered consciousness within the four hours, but both complained of headache, vertigo and burning/tearing eyes. The third man, who had been exposed for 25 minutes, was still only partly conscious on admission. Elevated serum ALT and AST levels, indicating that some damage had occurred in the liver, were observed up to three days post-exposure by which time they had returned to within the normal range. Blood and urine samples were taken for analysis for trichloroethylene and its metabolites at intervals until the men were discharged between five and thirteen days post-exposure. From the toxicokinetic data derived in this case, and taking into account previously published data on the relationship between air and blood concentrations of trichloroethylene in exposed volunteers, it was calculated that the men had been exposed to about 15,000 mg/m^3 (2,800 ppm) of trichloroethylene. Clearly, the reliability of this estimate of the exposure level is questionable and the value reached seems to be low in relation to the rapid loss of consciousness observed.

A clinical study has been carried out on ten men who suffered from acute trichloroethylene intoxication, due to accidental spillage of the material (Cotter, 1950). Most of the men became unconscious for a short period. Later effects noted included cramps, diarrhoea, headaches, and lower back pain, which persisted for one month or more. Blood tests at intervals over a two-month period following the incident gave no indication of any impaired liver function, using the insensitive cephalin flocculation test. None of the men showed any signs of jaundice. Hypercalcaemia and hyperglobulinaemia were noted in several of the men, and transient haematuria in one subject, one week after exposure. Apart from this instance, all urine samples were normal; there was no indication of any nephrotoxicity.

In another case, a 34-year old male was exposed for eight hours to trichloroethylene (99.5% pure) while cleaning computer ribbons for recycling (David et al., 1989). He wore gloves but no respirator and the 800 m^3 room containing the 7.5 litre vessel of trichloroethylene was poorly ventilated. There were no symptoms of toxicity until the next day, when the subject was drowsy and developed a distaste for alcohol and nicotine. Over the next few days, he developed symptoms of acute renal failure and was admitted to hospital three weeks after the exposure. Histological examination of tissue obtained via a needle biopsy showed acute interstitial nephritis with secondary tubular necrosis and tubular obstruction by intraluminal casts. The patient eventually made a full recovery. Although there was no exposure of the subject to any

other known nephrotoxicants, this single case provides very limited evidence that acute exposure to trichloroethylene may cause acute renal failure. The authors cited one other case of acute renal failure following use of trichloroethylene by a metal degreaser, reported by Gutch in 1965.

Sudden death has also been reported in four men occupationally exposed to trichloroethylene during the operation of a degreasing tank (Keinfeld and Tabershaw, 1954). Trichloroethylene levels were known to have been sufficiently high to produce symptoms of CNS disturbances, but all the men had continued work despite repeated complaints of drowsiness, dizziness and vomiting. Trichloroethylene concentrations were reported to be between 200 and 8,000 ppm in the working area of one individual. No details of trichloroethylene exposure were reported for the other 3 workers. All died within several hours of leaving work, and no significant autopsy findings were recorded. It was suggested that death was produced by ventricular fibrillation, due to the sensitising effect of trichloroethylene on the action of endogenous catecholamines on the myocardium. There are a few other reports of isolated cases of sudden death from cardiac arrest following exposure to high concentrations of trichloroethylene, either occupationally (Bell, 1951) or following repeated deliberate exposure (e.g. trichloroethylene sniffing at work (James, 1963). Cardiac toxicity, sometimes causing death, has been reported in a number of other cases of "recreational" solvent abuse and attributed to trichloroethylene (e.g. Delepoulle et al., 1989; Mee and Wright, 1980). Frequent repolarisation disorders, reminiscent of ischaemic myocardial lesions, have been suggested as the aetiology of the condition but in many of these cases, there is either clearly mixed exposure or there is insufficient evidence on the chemical nature of the solvent used.

Cranial neuropathy was reported in a young woman accidentally exposed to several thousand ppm of trichloroethylene for an unknown period (Sagawa et al., 1973). The subject was unconscious for two hours. On recovery, complete loss of sensation in the trunk and lower extremities was noted, together with disturbances of vision, interpreted as an effect on the optic nerve. Nerve conduction velocity studies revealed the absence of peripheral neuritis and it was suggested that the effects observed were due to lesions in the spinal cord. The sensory loss was persistent, with only a slight recovery being noted over the nine-month observation period. Much milder effects were noted in a second woman who was unconscious for only one hour.

Another case of trigeminal sensory loss has been noted after accidental exposure of an individual to high concentrations of trichloroethylene, for a few minutes, whilst investigating a leak in a degreasing machine (Feldman, 1970). Nausea, vomiting and giddiness occurred, but not anaesthesia. Extensive sensory loss occurred over the face, with numbness also being noted in the mouth and pharynx. This subject also experienced blurred vision, suggesting involvement of the optic nerve. Complete numbness over a large area of the face was maintained for about three months, with areas of hyperanalgesia for over a year. A slow improvement in function of the facial nerve was noted over this period, with recovery being essentially complete after eighty weeks.

The likelihood that the subjects in the above two studies were exposed to those breakdown products of trichloroethylene implicated in effects on the trigeminal nerve (Mertens, 1993; Saunders, 1967; Sax and Lewis, 1989) cannot be assessed.

Use of trichloroethylene as an anaesthetic

The use of trichloroethylene as an anaesthetic has been mainly limited to short procedures requiring light anaesthesia but good analgesia. Inhalation of concentrations in the range 5,000 to 20,000 ppm has been used to produce light anaesthesia (Reynolds et al., 1989). Blood levels required to produce anaesthesia have been stated to be of the order of 10 mg of trichloroethylene

per 100 ml (100 μg/ml); these would result from sustained inhalation of air containing 7,000 ppm of trichloroethylene (Langton-Hewer, 1975).

Trichloroethylene has been regarded as a relatively safe anaesthetic. In general, no untoward effects occur after recovery from unconsciousness, provided that the gas is not used in a closed-circuit system where the soda-lime used to eliminate carbon dioxide may result in decomposition of the trichloroethylene to toxic products, including dichloroacetylene and possibly also phosgene and carbon monoxide. This restriction has limited its use as an anaesthetic.

Hepatotoxicity has not been a problem, cases of serious effects on liver function being very rare following trichloroethylene anaesthesia (Hunter, 1962). In one study to investigate the effect of trichloroethylene on liver function (using a flocculation test), some evidence of impairment was noted in most of the patients (34/35) 24 hours after the operation. This was however transient, and all tests were normal after two weeks (Armstrong, 1944). There is a brief case report of severe liver necrosis in a patient subjected to prolonged (41/2 hour) use of trichloroethylene as an anaesthetic (Herdman, 1945).

Cardiac arrhythmias have been reported in subjects undergoing trichloroethylene anaesthesia. In one study the electrocardiogram (ECG) was recorded throughout the 30 to 90 minute period of anaesthesia (Barnes and Ives, 1944). Relatively minor arrhythmias (principally sinus bradycardia and auriculoventricular nodal rhythm) were recorded in the early stages of anaesthesia. Similar effects have been noted with other anaesthetics, and it was suggested that they reflect increased vagal tone. Of greater significance was the development of multifocal ventricular tachycardia in about 10% of patients during the later, deeper stages of anaesthesia. Other studies have reported ventricular tachycardia, with multiple extrasystoles, in a significant proportion of patients both with and without pre-existing myocardial degeneration, whilst undergoing trichloroethylene anaesthesia (Waters et al., 1943). However, although cases of cardiac arrhythmia have been reported during trichloroethylene anaesthesia and fatalities have occurred, possibly following serious ventricular arrhythmias, such events are rare. A review of the literature in 1957, about 16 years after the widespread introduction of the substance as an anaesthetic, identified only thirty-four cases of cardiac arrest occurring under trichloroethylene anaesthesia (Norris and Stuart, 1975). Furthermore, it has been suggested that eleven of these were due to causes other than trichloroethylene (Boulton and Sweet, 1960). The latter authors reviewed reports of about 70,000 cases where trichloroethylene anaesthesia had been used and revealed only two deaths that were likely to have been caused by the anaesthetic. No cases of liver damage were noted.

There are a few reports of other serious side effects following trichloroethylene anaesthesia (Eichert, 1936; Endergy, 1944; Firth and Stuckey, 1945). In the past there were several reports of cranial neuropathies, principally affecting the trigeminal nerve, but these occurred when the gas was used in a closed-circuit system with soda-lime to absorb carbon dioxide. These trigeminal palsies were believed to be due to formation of trichloroethylene decomposition products, particularly dichloroacetylene, after reaction with the alkaline soda-lime. Such neuropathies have not been reported in situations where soda-lime was not used.

Studies in volunteers under controlled conditions

- Studies to investigate performance in behavioural tests

There are several reports of investigations of the acute effects of trichloroethylene in volunteers, under controlled conditions. It is known that trichloroethylene can affect CNS function and these studies have, in the main, concentrated on the effect of exposure to relatively low levels of trichloroethylene on the performance of certain psychological or psychomotor tests. Anaesthetic

grade trichloroethylene was usually used in the studies, which are summarised below. For most of these studies, it is not clearly stated whether or not the subjects had any previous experience of exposure to trichloroethylene. In addition, it is sometimes not clear, in those studies in which a separate control group was not used, whether the subjects were tested in the absence, as well as the presence, of trichloroethylene so as to provide their own control data.

No significant impairment in performance of behavioural tests was observed in a study in which the effects of exposure to two concentrations of trichloroethylene (50 and 110 ppm) were investigated in nine subjects (Stewart et al., 1974). There were two 4-hour periods of exposure to trichloroethylene, separated by a l½-hour lunch break. Each individual was given a comprehensive medical examination both prior to the first exposure and after the last exposure. Electroencephalograph (EEG) tracings were recorded throughout the exposure period. Any reported symptoms of toxicity were noted.

A range of six tests was conducted. These comprised a complex reaction time test, a tachistoscopic perception test (ability to reproduce the pattern of images seen on a screen), a digit span test (ability to repeat a list of numbers given orally in the correct or reverse order), a ringer dexterity test (placing pins in holes), the Flannigan co-ordination test (ability to do pencil tracings) and a digit inspection test (speed taken to delete a given number from a list of random numbers). Each volunteer performed these tests soon after entering the exposure chamber in the morning, and l½ hours prior to termination of exposure in the afternoon.

No significant abnormalities were noted in any subject during the medical examination, prior to or after the test, nor were any abnormalities detected in the EEG tracings during the exposure period. No significant impairment was noted in any of the tests during the trichloroethylene exposure at either concentration. A slight impairment was noted in the performance of the Flannigan co-ordination test in two individuals at 110 ppm, but only in the mornings. In view of the lack of effects in the afternoon, and in performance of the other tests, this was not believed to be significant. However there was fairly high individual variability in most of the tests.

Thus in this study no significant reduction in performance was noted in a range of behavioural tests when healthy volunteers were exposed to 50 or 110 ppm of trichloroethylene for eight hours.

In another study, twelve volunteers (students) were exposed in groups of three to 0, 27, 81 or 201 ppm trichloroethylene for 4 hours (Nomiyama and Nomiyama, 1977). The subjects were examined every hour during exposure for odour perception, eye irritation, sore throat, dryness of throat, nasal obstruction, rhinorrhoea, drowsiness, fatigue, headache, dizziness, palpitation, neuralgia and numb fingers, blood pressure, pulse rate, respiration rate, flicker fusion frequency, and two-point discrimination. A number of toxicokinetic parameters were also investigated: the results of these investigations are included in the relevant sections above.

The volunteers noticed the smell of the substance at 27 ppm but within three hours had lost sensitivity to the smell even at 201 ppm. At 27 ppm and above, “irritation” of mucous membrane and eyes and drowsiness were reported and, after two hours of exposure to 81 ppm, headaches also occurred. After four hours at 201 ppm, dizziness and skin irritation were also reported. No significant changes were seen in flicker fusion frequency, two-point discrimination or respiratory rate. There is no indication in the study report of the severity of any of the reported effects, which are all of a subjective nature. It is noteworthy that "irritant effects" were reported in this study to occur at 27 ppm of trichloroethylene, but have not been reported in other volunteer studies even at considerably higher exposure levels.

Eight male volunteers were subjected to a range of psychomotor tests whilst exposed to 0, 100, 300 or 1,000 ppm of trichloroethylene, using a breathing tube, for two hours (Vernon and

Ferguson, 1969). Each subject performed six different tests to measure visual-motor function. All the tests were conducted three times during each two-hour session, with the exception of the pegboard test for manual dexterity, which was performed only immediately prior to and immediately after exposure to 0 and 1,000 ppm of trichloroethylene. Symptoms of CNS depression (light-headedness, dizziness and lethargy) were noted in some of the subjects when exposed to 1,000 ppm and in one individual at 300 ppm. No symptoms were noted by any of the volunteers when exposed to 100 ppm of trichloroethylene. Significant effects on performance were noted at 1,000 ppm in both a perception test (Howard Dolman) and a test for steadiness. Five subjects also showed reduced performance in the pegboard test at this concentration, but the other three subjects showed some improvement in performance. No effects were noted on performance of any of the tests at 300 ppm. Thus, in this study some symptoms of CNS depression, together with changes in performance in certain psychomotor tests, occurred in subjects exposed to 1,000 ppm trichloroethylene for two hours, but exposure at 300 ppm for the same period of time caused no significant effects.

Similar results were obtained in a later study by the same investigators, though in this case, slight effects (not statistically significant) on performance in the series of standard visual-motor tests were noted at 300 ppm trichloroethylene (Ferguson and Vernon, 1970). In this study, alcohol (0.5 ml/kg) was shown to potentiate the effects of exposure to 1,000 ppm, but not 300 ppm, of trichloroethylene; marked subjective symptoms were noted (dizziness, inability to concentrate, floating sensation) together with greater changes in performance of the visual-motor tests. Alcohol alone, at the dose given, had no significant effect.

In another, briefly reported study, the effects of exposure to trichloroethylene on psychophysiological efficiency have been investigated in a group of six young adult males who were not normally exposed to the substance (Salvini et al., 1971). The volunteers underwent a series of tests (to evaluate perception, memory, complex reaction time and manual dexterity) on two separate days on one of which they were exposed to trichloroethylene at around 110 ppm (measured concentrations ranged from 90 to 130 ppm) for two 4-hour periods separated by a 1.5-hour interval. There was no exposure to trichloroethylene on the other day. Tests were performed at 08.30 h and 18.00 h on each day. All the subjects complained about the odour of the substance. No clinical disturbances in motor function, co-ordination, equilibrium or behaviour patterns were apparent. The volunteers reported slight dizziness and transient eye irritation during some periods when exposures rose towards the top of the range. Statistically significant decreases in performance ability in all the psychophysiological tests, particularly the more complex tasks, were reported. However, although this report seems to indicate, in contrast to the studies summarised above, that impairment of performance can be induced by short-term exposure to trichloroethylene at concentrations up to 130 ppm, only a statistical analysis of the results was given, not the actual data, and the volunteers were clearly aware of exposure to the substance. The study was repeated with six workmen who regularly worked with trichloroethylene, and were thus more used to its odour. Similar reductions in performance ability were recorded but, again, no actual results were given.

Effects on psychomotor performance had been noted previously in a very limited study involving only one subject (Stopps and McLaughlin, 1967). He performed a range of tests to measure pyschomotor performance whilst exposed to 0, 100, 200, 300 or 500 ppm of trichloroethylene for about 23/4 hours. Lavender oil was used to mask the smell of the trichloroethylene. The signs of toxicity reported at 500 ppm were all of a subjective nature and included “mild irritation” to the upper respiratory tract, a dull and woolly-headed feeling and somnolence. The effects were reversed within 15 minutes of leaving the exposure chamber. The only effect reported at lower

concentrations was a slight tendency to somnolence. No effects were noted on performance in any of the behavioural tests at 100 ppm, but progressive changes were noted at 300 ppm and above.

In another experiment, twelve volunteers were subjected to a range of behavioural tests whilst exposed to 50 ppm of trichloroethylene for an unknown period of time (probably three hours, as below) (Winneke et al., 1976). No details of the exposure chamber used, nor of the method of generating the trichloroethylene atmosphere were given. The investigations included reaction time test, tapping test (measurement of hand/arm speed) and the pursuit rotor test (precision of pursuit tracking by measuring time on target). Control values were obtained for each individual under the same conditions, but in the absence of trichloroethylene. No significant differences from control results were noted in the performance of any of these tests when the subjects were exposed to 50 ppm of trichloroethylene.

The effect of exposure to both 50 ppm of trichloroethylene and alcohol was investigated in a second series of studies by the same investigators. The volunteers were given ethanol (600 mg/kg) in orange juice one hour after exposure started. They were then exposed to trichloroethylene for a further two hours, and then performed the same series of behavioural tests as described above. Blood alcohol levels at that time were about 30 mg/100 ml. Significant ($p < 0.05$) changes in performance were recorded in both the pursuit rotor test and the tapping test (reduced maximum "tapping time") under these conditions. However the alcohol alone produced a similar effect on the pursuit rotor test, but not on the tapping test.

Thus, in this series of studies, exposure to 50 ppm of trichloroethylene produced no effect on performance of a range of behavioural tests. Exposure to moderate amounts of alcohol resulted in significant effects on performance of the pursuit rotor test but not the other tests. This was not potentiated by simultaneous exposure to 50 ppm of trichloroethylene. Exposure to both trichloroethylene and alcohol produced some changes in the maximum "tapping time". However in view of the lack of any potentiation in any of the other measurements, no significance can be given to this observation.

The effects of an exposure to 150 or 300 ppm of trichloroethylene on performance of a range of behavioural tests have been investigated in healthy volunteers, and the results compared with the results following the administration of moderate amounts of alcohol (Ettema and Zielhuis, 1975; Ettema et al., 1975). In this study a total of 47 young adult males (aged between 19 and 27 years) were randomly divided into three groups of 15 or 16 individuals. They were exposed to 0, 150 or 300 ppm of trichloroethylene for 2½ hours, during which time they were subjected to a range of behavioural tests. These consisted of binary choice tasks (separate tests using auditory and visual signals), the Bourdon-Wiersma test (identifying those groups of dots on paper that contain four or five dots), identification test (detecting small differences in columns of similar figures or words) and a memory test (repeating lists of common words). Any signs of toxicity reported during the exposure period were recorded, and heart rate was monitored continuously using a cardiotachometer.

No significant differences were noted in performance of these tests in any group except for a slight, but not statistically significant, change in performance of the Bourdon-Wiersma test noted in subjects exposed to 300 ppm of trichloroethylene, nor were there any differences in heart rate. No signs of toxicity were reported.

Thus, in this study, inhalation exposures of up to 300 ppm of trichloroethylene for 2½ hours had no significant effect on the performance of a range of behavioural tests. In contrast, in separate experiments, oral doses of alcohol (20 g, giving blood levels of about 45 mg/100 ml) resulted in definite impairment of performance in both the binary choice tests and the Bourdon-Wiersma test.

Further work has been carried out by this group of investigators on the combined effects of trichloroethylene and alcohol (Windemuller and Ettema, 1978). In this study, the behavioural tests were limited to a binary choice test using visual stimuli and the pursuit rotor test (following the movement of an illuminated point). These tests were performed in a separate room immediately after exposure ceased, rather than during exposure, as in the previous studies.

In one experiment a group of 24 male students (age 19-26 years) was subdivided into four groups of six. One served as a control group, and the others were exposed separately to alcohol alone, 200 ppm of trichloroethylene, or 200 ppm of trichloroethylene plus alcohol; each group spent 21/2 hours in the exposure chamber. The alcohol was given as a 35% solution in orange juice, at a dose level of 350 mg/kg, 50 minutes prior to the end of the exposure. Physiological parameters monitored during the exposure included breathing rate, using a nasal thermistor, and heart rate.

Blood alcohol levels (measured 30 minutes after leaving the exposure chamber) in those exposed to trichloroethylene were the same as in those given alcohol alone (27 $\pm$ 7 mg/100 ml and 27 $\pm$ 4 mg/100 ml, respectively). Blood levels of trichloroethylene were, however, considerably higher in those co-exposed to alcohol than in those exposed to trichloroethylene only (reported to be 63 $\pm$ 12 μg/litre and 29 $\pm$ 3 μg/litre, respectively) indicating that trichloroethylene metabolism had been markedly inhibited by simultaneous exposure to alcohol.

No significant impairment was noted in the performance of either test in any of the exposed groups as compared to the controls.

In a second experiment, 15 male volunteers were used, each serving as his own control. Each was subjected, in random order, to similar trichloroethylene exposure conditions to those described above, with a two-week break between exposures. In this study the alcohol was given ten minutes prior to the end of the 2½-hour period in the exposure chamber. Blood alcohol levels were measured 10 and 20 minutes after leaving the exposure chamber. These were 48 $\pm$ 9 mg/100 ml and 41 $\pm$ 6 mg/100 ml at 10 and 20 minutes respectively in those not exposed to trichloroethylene and were not significantly different in those also exposed to trichloroethylene.

For all of the exposure conditions, there were no significant differences in heart or breathing rates nor was there any impairment in performance of the binary choice test. A slight decrease was noted in performance of the pursuit rotor test in subjects with blood alcohol levels of about 45 mg/100 ml following exposure to alcohol alone, the mean values being 104.9 $\pm$ 41.2 seconds compared with 110.6 $\pm$ 26.4 seconds in controls. This change was more marked in those simultaneously exposed to 200 ppm of trichloroethylene (mean value 93.4 $\pm$ 37.2 seconds). However there was much individual variability and the decrease was not statistically significant. Administration of the alcohol was shown to markedly inhibit the metabolism of trichloroethylene. No impairment in performance was noted in subjects exposed to 200 ppm of trichloroethylene alone.

A study of 15 men subjected to a range of tests to measure psychological function whilst being exposed to trichloroethylene was too poorly reported to assess the significance of the results (Gamberale et al., 1976).

- Summary of effects of trichloroethylene on performance in behavioural tests

Significant effects on performance were noted in a range of behavioural tests to measure visual motor effects when volunteers were exposed to 1,000 ppm of trichloroethylene for two hours. Signs of CNS depression (dizziness, light-headedness, lethargy) were also noted at this exposure level. No significant effects on performance, nor any signs of toxicity (including effects on heart

rate) were observed in subjects exposed to trichloroethylene for two to eight hours at 300 ppm or below.

The combined effects of trichloroethylene and alcohol have been investigated by several workers. Exposure to alcohol alone (sufficient to give blood levels of 30 to 45 mg/100 ml) resulted in reduced performance in certain behavioural tests. Simultaneous exposure to alcohol (as above) and trichloroethylene (at 200 ppm and above, for 2½ hours) gave effects on performance that were more marked than those observed with either substance alone. There was evidence that the metabolism of trichloroethylene was inhibited by simultaneous exposure to these dose levels of alcohol.

Other studies in volunteers

To investigate the effects of trichloroethylene on cardiac rhythm, studies have been carried out on 20 healthy male volunteers, under controlled conditions (Konietzko et al., 1975). The average age of the men was 27 years. Each subject was exposed, while sitting down, to 95 ppm of trichloroethylene for four hours in an exposure chamber, during which time the ECG was monitored continuously. Abnormalities during the exposure period were noted in one individual. These were regular ventricular extrasystoles, which appeared after about 15 minutes of exposure, and continued for about one hour. The ECG then returned to normal. No changes were noted in any subject during comparable assessments in the absence of trichloroethylene.

The effect of trichloroethylene on optokinetic nystagmus has been investigated in twelve volunteers (Kylin et al., 1967). Slight changes were observed after a two-hour exposure to the single concentration used (1,000 ppm) indicating some effects on the CNS.

In another study, the effect of trichloroethylene on serum marker enzymes for liver dysfunction was investigated (Konietzko and Reill, 1980). Twenty healthy volunteers were exposed to 95 ppm of trichloroethylene for four hours. Serum enzyme levels were measured immediately prior to exposure, and at 0, 4 and 20 hours post-exposure. No effects were seen, suggesting that no liver damage had occurred during the short time period of the experiment.

Subjective signs of CNS depression were reported when groups of 5 or 6 volunteers were exposed to 50 and 100 ppm of trichloroethylene for six hours (Ertle et al., 1972). They consisted of fatigue and decreased ability to concentrate, and were experienced by all subjects at both concentrations. Headache was reported by one subject at each exposure level. However, this study was intended primarily to investigate the metabolism of trichloroethylene, and no attempt was made to qualify these observations. No controls were used, and the reported effects may have been due to the enforced sedentary period throughout the exposure. In view of the limited nature of the toxicological observations in this study, and the fact that similar effects have not occurred in other studies in volunteers (above) no conclusions can be drawn as to whether the effects observed were related to trichloroethylene exposure or to other factors.

Summary of acute inhalation toxicity of trichloroethylene in humans

The main signs of toxicity associated with acute exposure of humans to trichloroethylene are those of CNS depression, exposure to high concentrations (several thousand ppm) resulting in narcosis, which may be fatal if respiratory failure or severe cardiac effects occur. Overt liver or kidney damage has only rarely been observed, but biochemical signs of transient liver damage have been observed following accidental exposures to unknown, but possibly very high, concentrations of trichloroethylene. When recovery from narcosis occurs, it is usually complete with no serious sequelae, and trichloroethylene has been widely used as an anaesthetic at

concentrations of about 5,000 to 10,000 ppm for operations of short duration. Effects on cardiac rhythm, including potentially serious multifocal ventricular tachycardia, have been recorded during trichloroethylene anaesthesia, but cases of cardiac arrest are very rare. Cranial neuropathies have occurred in the past when closed-circuit anaesthetic systems were used, but these are considered most likely to be due to decomposition products of the trichloroethylene by the action of alkaline soda-lime used to absorb carbon dioxide. There have been very few reports of cranial neuropathy following accidental exposures to trichloroethylene. The possibility of breakdown products being present in these cases cannot be assessed.

Studies in volunteers have concentrated mainly on investigating the effects of exposure to relatively low levels of trichloroethylene on performance of certain behavioural tests. Marked changes in performance of a range of visual-motor tests were observed following exposure to trichloroethylene for two hours at 1,000 ppm. These changes were potentiated when there was concurrent exposure to alcohol. No significant effects have been noted in this or several other studies with exposures at 300 ppm or below for two to eight hours. In none of these studies were significant signs of CNS depression, or any other toxic signs) noted at 300 ppm or below but some subjective effects (dizziness, light-headedness, lethargy) were reported following exposure for two hours at 1,000 ppm. Co-exposure to alcohol (up to 47 mg/100 ml) caused increased blood concentrations of trichloroethylene, but the changes that the alcohol alone caused in performance were not potentiated by co-exposure to trichloroethylene at concentrations of $\leq$ 300 ppm.

There is no evidence from any of the data from accidents, use of trichloroethylene as an anaesthetic or studies in volunteers that pulmonary toxicity occurs in humans following single inhalation exposures to trichloroethylene.

Thus, there is evidence that exposure of humans to 300 ppm of trichloroethylene for a single period of two to eight hours can be expected to give rise to no toxicologically significant effects.

Oral

There are a few reports of acute poisoning following oral ingestion of trichloroethylene. However, the amount of useful data is limited because of the reliance on individual case reports for data on the acute oral toxicity of trichloroethylene in humans and the very variable extent of investigation and quality of the reports.

Single oral intakes of quite small volumes of trichloroethylene ($\leq$ 20 ml, equivalent to about 450 mg/kg body weight, or less) are associated with CNS effects varying from headache and slight confusion to coma, but there are few other effects and recovery is uncomplicated (Morreale, 1976; Naish, 1945; Secchi et al., 1968; Stephens, 1945; Todd, 1954). The main effects of ingestion of higher volumes of trichloroethylene ($\geq$ 50 ml, equivalent to about 1,100 mg/kg body weight, or more) are on the CNS and the heart (Calvet, 1959; Forboese, 1943; Keinfeld and Tabershaw, 1954; Koppel et al., 1988; Meyer, 1966 and 1973; Migdal et al., 1971; Nakajima et al., 1987; Pistelli et al., 1990; Roche et al., 1958; Schneider and Klug, 1982; Tomasini, 1976; Wells, 1982). Cardiac effects reported include tachycardia and ventricular extrasystoles. There is limited evidence associating ingestion of 50 ml or more of trichloroethylene with liver and/or kidney toxicity. Death, attributed to ventricular fibrillation, has occurred as a consequence of ingestion of 50 ml, but patients have recovered even after ingestion of up to 200 ml of trichloroethylene (around 4,500 mg/kg body weight).

Dermal

There is only one report of the effects of a single dermal exposure to trichloroethylene. This report describes the case of a 47-year-old woman who received a single 2½-hour exposure to trichloroethylene predominantly by the dermal route when doing a new job: degreasing X-ray tubes by immersion into a tank fitted with vapour condenser coils (Lockey et al., 1987). The woman did not wear a respirator but wore gloves (stated to have many tears) and trichloroethylene splashed and spilled over their rims. During the next few days, the woman developed fatigue and swelling of the hands and forearms with a pruritic macular rash over the areas exposed to trichloroethylene. Despite hospitalisation and treatment her condition worsened over the next three months as severe erythema, pain and swelling of her legs, diffuse arthralgias and proximal muscle weakness developed. She died six months after the exposure to trichloroethylene. The autopsy revealed changes characteristic of progressive systemic sclerosis. Acute symptoms of trichloroethylene exposure were not seen and the use of condenser coils in the tank suggests that inhalation exposures were probably relatively low, but there were no examinations of blood or urine for trichloroethylene and its metabolites. Whether or not the disease in this case can be causally associated with exposure to trichloroethylene cannot be established.

4.1.2.2.3 Summary of acute toxicity

The main toxic effect associated with acute inhalation exposure of humans to trichloroethylene is CNS depression. Exposure to high concentrations (several thousand ppm) causes narcosis, which may be fatal. Overt liver or kidney damage has only rarely been observed, but biochemical signs of transient liver damage have been seen following exposures to very high concentrations of trichloroethylene. When recovery from narcosis occurs, it is usually complete, illustrated by the fact that trichloroethylene has been widely and largely successfully used as an anaesthetic at concentrations of about 5,000 to 10,000 ppm for operations of short duration. Effects on cardiac rhythm, including potentially serious multifocal ventricular tachycardia, have been recorded during trichloroethylene anaesthesia, but cases of cardiac arrest are very rare. Cranial neuropathies have been reported, but are considered likely to be due to decomposition products of the trichloroethylene.

In volunteers, marked changes in performance of a range of visual-motor tests were observed following exposure to trichloroethylene for two hours at 1,000 ppm. These changes were potentiated when there was concurrent exposure to alcohol. No significant effects have been noted in this or several other studies with exposures at 300 ppm or below for two to eight hours.

There is no evidence from any of the data from accidents, use of trichloroethylene as an anaesthetic or studies in volunteers that pulmonary toxicity occurs in humans following single inhalation exposures to trichloroethylene.

Thus, there is evidence that exposure of humans to 300 ppm of trichloroethylene for a single period of two to eight hours can be expected to give rise to no toxicologically significant effects.

CNS depression is also the main toxic effect of single oral doses of about 450 mg/kg bodyweight of trichloroethylene in humans and full recovery is usual at these dose levels. Doses of 1,100 mg/kg or more cause CNS depression and also effects on the heart (e.g. tachycardia and ventricular extrasystoles). Fatal ventricular fibrillation has occurred following ingestion of about 1,100 mg/kg, but there has also been recovery from doses four times higher.

There is no useful information concerning acute dermal exposure of humans to trichloroethylene.

In animals the main signs of toxicity following inhalation exposure are, as in humans, those typical of CNS depression, seen following exposure at 2,000 ppm or more for 4 hours. Full anaesthesia is induced at similar trichloroethylene levels in animals (5,000 ppm for 4 hours) as it is in humans. Also as in humans, there are indications of transient hepatic dysfunction in trichloroethylene-exposed animals but the evidence for potential nephrotoxicity is even less in animals than in humans following single inhalation exposure. There was no indication of significant effects on rodent behaviour at trichloroethylene levels of 500 ppm for 4 hours or 400 ppm for 6 hours, which is very similar to the "no-effect level" in human volunteers (300 ppm for 2 to 8 hours). Cardiac effects have also been reported in both animals and humans. In rabbits, cardiac hyperreactivity to catecholamine stimulation was apparent following exposure to $\geq$ 6,000 ppm, but not 2,000 ppm, of trichloroethylene for 1 hour. In general, mice were more sensitive to the toxic effects of single inhalation exposures to trichloroethylene than rats and, similarly to humans, in all species in which there was co-exposure to alcohol, the effects of trichloroethylene were enhanced. In mice, subtle effects were seen in the lung (vacuolation of Clara cells) at exposure levels as low as 20 ppm; however, there is evidence that this effect is related to the way in which the mouse metabolises trichloroethylene, so this effect is thought unlikely to be of relevance to humans.

Trichloroethylene was of low acute oral toxicity in animals and the only signs of toxicity were CNS depression and effects on the liver. There were no useful data from acute dermal studies in animals.

Overall, the no effect level for single inhalation exposure of humans to trichloroethylene is around 300 ppm for up to eight hours, which, apart from the specific effects induced in mouse lung following exposure at lower concentrations, is very similar to that which can be derived for animals. Simultaneous exposure to moderate amounts of alcohol has been shown to enhance the acute effects of trichloroethylene in humans, as it does in animals.

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin

Trichloroethylene (99.5% pure) has been shown to produce severe skin irritation when tested undiluted on rabbit skin using a 24-hour application period and an occlusive dressing (Duprat et al., 1976). In another study, marked irritation was recorded following application to rabbit skin (Smyth et al., 1969). No experimental details were given, but this series of studies on various substances is known to have been conducted using the standard Draize protocol. However, insufficient details are available for direct comparison with the European Union (EU) criteria for classification of substances in relation to skin irritancy.

The skin irritant effects of repeated dermal application of trichloroethylene have also been studied. The skin irritancy of a number of solvents has been assessed in a well-reported study using both animals and humans (Wahlberg, 1984). Six guinea pigs and four rabbits were exposed daily for 10 days. Twelve control rabbits and 22 control guinea pigs were used. Trichloroethylene ("extra pure", 0.1 ml) was applied undiluted to unoccluded skin sites on the test animals. Every 24 hours the skin was visually scored for erythema, oedema, fissuring and scaling and skin fold thickness was measured. Application of trichloroethylene caused visual erythema and oedema at the first 24-hour reading and on repeated exposure, all animals reacted

strongly with evidence of fissuring and scaling. Increases in skin fold thickness, relative both to pre-treatment values in the treated animals and to values in concurrently examined untreated animals, were seen in both species after repeated administration of trichloroethylene rising to more than 200% of pre-treatment/control values by seven days. In a separate study in rabbits, application of 0.1 ml of undiluted trichloroethylene to the skin caused erythema within 5 minutes and by 24 hours, skin fold thickness had increased to 150% of the pre-treatment value.

The skin irritancy of trichloroethylene has also been assessed in a study of several solvents conducted in female Dunkin-Hartley guinea pigs (Anderson et al., 1986). Ten µl of trichloroethylene in alcohol (trichloroethylene concentration unknown) or 5 µl of trichloroethylene in an aqueous solution (trichloroethylene concentration unknown) was applied, three times daily for three days, to a 1 cm^2 shaved area on the flank of guinea pigs which was left unoccluded. The test sites were assessed visually for signs of erythema and by palpation for swelling. Post-mortem biopsies were taken from both treated and untreated skin. They were examined for epidermal thickness and for histological evidence of infiltration by dermal inflammatory cells. Skin responses were compared with those of animals treated with 1, 2 or 4% solutions of a known skin irritant, sodium lauryl sulphate (SLS). Of the solvents tested, trichloroethylene was the most irritant (equivalent to 2% SLS) based on both time to response and extent of responses. Ethanol alone was not irritant.

Eye

Application of 0.1 ml of 99.5% pure trichloroethylene directly to the rabbit eye produced a mild-to-moderate conjunctivitis with some epithelial abrasions being noted on examination with fluorescein (Duprat et al., 1976). Microscopic examination on day seven revealed epithelial keratosis in the process of healing. The eye had returned to normal within two weeks. In another study, instillation of 0.1 ml trichloroethylene to the rabbit eye reportedly caused necrosis of 80-100% of the cornea, but insufficient data are available to evaluate this study fully (Smyth et al., 1969). Insufficient experimental data are available for direct comparison with the criteria for classification of substances in relation to eye irritancy.

4.1.2.3.2 Studies in humans

Skin

Trichloroethylene is clearly irritating to human skin. Repeated dermal contact with trichloroethylene may, as with other solvents which cause defatting, lead to roughening, chapping and erythema (Irish, 1963). Eczematous lesions, and also cases of generalised exfoliative dermatitis have been reported in workers exposed to trichloroethylene in liquid or vapour form (Schirren, 1971; von Wuthrich, 1977; Bauer and Rabens, 1974).

Immersion of a hand in liquid trichloroethylene caused a burning sensation and distinct pain towards the end of the 30-minute exposure period (Sato and Nakajima, 1978). Following removal of the hand from the solvent, a moderate erythema was observed on the back of the hand, which lasted for over an hour.

In a much earlier case report, a 28-year-old male developed skin lesions following exposure to trichloroethylene vapour after entering a drained degreasing tank to retrieve metal parts which had fallen into the tank (Maloof, 1949). It was estimated that he was in the tank for 15 to 20 minutes. He was admitted to hospital unconscious. Upon regaining consciousness he complained of blurred and double vision and a burning sensation of the skin. He was found to

have first degree chemical burns of both conjunctivas with an abrasion of the right cornea, and mild erythema of the skin on most of his body which worsened progressively until vesicular eruptions appeared. The lesions healed within 11 days and no residual effects were observed.

A 54-year-old male had been employed as a degreaser using trichloroethylene. An accident at the plant resulted in his face shoulders and chest being bathed in trichloroethylene (Nakajima et al., 1987). He immediately washed his hands and face with water, but opthalmodynia (pain in the eyes) developed.

A healthy human volunteer was repeatedly exposed to a number of solvents on the unoccluded skin on the flexor surface of the right forearm in order to study the ability of the substances to induce erythema (Wahlberg, 1984). Test sites were treated daily for 18 days with an unstated quantity of trichloroethylene. Skin fold thickness was measured and erythema recorded to assess irritancy. No increase in skin fold thickness was seen with 18 days treatment and no erythema was seen in readings taken 24 hours after each exposure. It is probable that there was rapid evaporation of the substance from the application site.

Eye

Virtually no specific data are available. In a secondary source of information it is stated that direct eye contact with liquid trichloroethylene may cause smarting and injury to the corneal epithelium with complete recovery usually within a few days, even in cases of extensive contact (Grant, 1974). In the case reported above, pain in the eyes occurred following an accident in which eye contact with liquid trichloroethylene or its vapour was possible (Nakajima et al., 1987).

4.1.2.3.3 Summary of skin and eye irritation

For neither skin nor eye irritation are there appropriate experimental data available for direct comparison with the criteria for classification. However, there are indications from human experience and studies in animals that both single and repeated dermal exposure to trichloroethylene can be irritating to the skin, as is to be expected given the defatting properties of the substance, and that it should therefore be classified as a skin irritant. Also, from the data which are available, it is apparent that trichloroethylene should be classified as an eye irritant.

4.1.2.4 Corrosivity

As is apparent from the studies cited in Section 4.1.2.3 and elsewhere, trichloroethylene is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

No skin or respiratory sensitisation studies have been conducted in animals

4.1.2.5.2 Studies in humans

Trichloroethylene is a widely used substance to which many people have had repeated inhalation and dermal exposure. There have been no reports of respiratory sensitisation and very few (see below) of apparent skin sensitisation.

In a report of a case in which a 21-year-old oriental male printing worker had severe dermal effects following exposure to sufficient trichloroethylene vapour to cause nausea and headache but not loss of consciousness, it is suggested that the mechanism was delayed hypersensitivity to trichloroethylene (Nakayama et al., 1988). The patient's skin lesions appeared during his first week in hospital and progressed to erythroderma with the face and eyes becoming severely oedematous. His skin was dry with extensive desquamation and cracking. In the third week, his hands and feet showed thick membranous desquamation and there was considerable loss of scalp hair. He made a full recovery in 10 weeks. Patch tests conducted four months after discharge from hospital revealed positive reactions considered to be delayed type hypersensitivity to both trichloroethylene (25% and 10% but not 5% in olive oil) and its metabolite trichloroethanol (5%, 0.05% and 0.005% in water). Another metabolite of trichloroethylene, trichloroacetic acid, gave negative results (5% in water). Ten control subjects, including 1 printer, gave negative patch test results to 25 or 5% trichloroethylene in olive oil and 5% trichloroethanol or trichloroacetic acid in water.

A 25-year-old woman who had worked for 8 years in a cutlery factory in which trichloroethylene was used, had for the past three years experienced intense itching all over her body at work with more recent appearance of erythematous lesions which became exfoliative (Conde-Salazar et al., 1983). The patient's symptoms regressed spontaneously when she was away from work. She had no systemic symptoms. She was patch tested with trichloroethylene (5% in olive oil) and a red scaly reaction was observed. Twenty control subjects did not respond. This individual was again challenged with trichloroethylene after some months during which she had been symptom free. She was placed in a room and exposed to trichloroethylene at nearly 100 ppm. Four to five hours into the exposure period she developed skin lesions on the face, hands and rest of skin with vesiculopustular lesions on the sides of the neck, spreading to the trunk. No trichloroethylene metabolites were detected in her blood or urine. General laboratory tests and a clinical and neurological examination were normal. Biopsy of one lesion revealed spongiform pustules in the subcorneal layer, some oedema and mixed perivascular infiltration in the upper dermis. IgE levels were elevated but no IgE antibody to an albumin-trichloroethylene complex was detected. Serum IgG, IgA, IgM and complement levels were normal.

A further challenge test was carried out at an unspecified time later when the woman was again asymptomatic. In this test the right leg only was exposed to a trichloroethylene-saturated atmosphere to exclude potential complications arising from inhalation exposure. Again, erythema appeared after a few hours, and later there was exanthema on the trunk and in the flexures lasting for a few hours.

There have been no reports of respiratory sensitisation in humans. Given the large number of people that have been exposed to trichloroethylene by the inhalation route and considering the general toxicological characteristics of trichloroethylene, all the evidence indicates that this substance is not a respiratory sensitiser.

4.1.2.5.3 Summary of sensitisation

There are no data available from studies in animals on the potential of trichloroethylene to cause either skin or respiratory sensitisation. In humans, there have been a few cases of individuals exposed to trichloroethylene apparently developing skin sensitisation to the substance, but the sparsity of such cases and the extensive use of trichloroethylene suggest that skin sensitisation is a highly idiosyncratic reaction and the substance should not be classified as a skin sensitiser.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

The toxicity of trichloroethylene following repeated or continuous administration has been extensively studied in a number of species following exposure by inhalation and by the oral route. However, no standard 28-day or 90-day studies with a full range of observations are available.

Inhalation

The effects of repeated inhalation exposure to trichloroethylene have been studied most extensively in the rat with animals being repeatedly or continuously exposed for periods of up to about eight months. Some toxicity data are also available from the longer studies conducted to investigate the carcinogenicity of trichloroethylene. Some studies have been conducted specifically to investigate the pulmonary toxicity, neurotoxicity and effects on hearing of trichloroethylene. These are addressed in discrete sections, below.

Wistar rats (6 male and 6 female in each exposure group) survived exposure to 0 or 3,000 ppm of trichloroethylene (7 hours/day, 5 days/week) for five weeks (Adams et al., 1951). There were no substance-related deaths. The only signs of toxicity noted were decreased weight gain, and some CNS disturbances, particularly from the second week onwards (excitability, hyperactivity and disturbances in equilibrium). Significantly increased relative liver and kidney weights (liver, 25% increase in males and 60% in females; kidney, 20% in males and 40% in females) were recorded at necropsy, but there were no histological changes in these organs, and there were no weight changes in lungs, heart, spleen and testes. Groups of 30 rats (15 male and 15 female) were also exposed to 0, 200 or 400 ppm of trichloroethylene (7 hours/day, 5 days/week) for about eight months. There were no deaths and no behavioural effects or signs of CNS depression were recorded. Body weight gain was slightly reduced in male rats exposed to 400 ppm of trichloroethylene and relative liver and kidney weights were increased (liver ~ 12% in both sexes, $p < 0.01$; kidney ~ 10% in both sexes, $p = 0.02$). There were no microscopic changes in liver or kidney. The no-effect level in this study was 200 ppm.

In another early study, using an unspecified strain of rat, some deaths occurred when the animals were exposed to 500 to 3,000 ppm of trichloroethylene (6 hours/day, 5 days/week) for six months (Taylor, 1936). Signs of slight narcosis were seen at 2,000 ppm, but all animals survived. No effects were noted on growth rate or on haematological parameters or clinical chemistry. At necropsy, some evidence of congestion of liver and kidneys was noted in animals that died at 3,000 ppm. No organ damage was observed at 2,000 ppm following gross and microscopic examinations. All rats survived exposure up to 5,000 ppm of trichloroethylene (6 hours/day, 5 days/week) for a shorter period (8 to 11 exposures). Deep narcosis occurred at 5,000 ppm, and slight drowsiness at 2,000 ppm.

No signs of toxicity were noted in rats exposed to 500 or 1,000 ppm of trichloroethylene (18 hours/day) for 90 days, 771 ppm (8 hours/day, 5 days/week) for six weeks or 35 ppm (24 hours/day) for 90 days (Nowill et al., 1954; Prendergast et al., 1967). In none of the studies were any effects seen on haematological parameters, nor were there any lesions apparent in heart, liver, lung, spleen and kidney on gross and microscopic examination.

In a study conducted specifically to investigate effects on the liver and kidney, rats were exposed to 372 ppm of trichloroethylene (30 minutes/day) for up to 120 days (Fonzi et al., 1967). Levels of serum enzyme markers of liver dysfunction (amino transferases) progressively increased from day 40 onwards. Some histological evidence of damage (hyperaemia) was seen in the livers of animals killed after 40 days. Liver lesions were more marked in animals killed after 120 days when some degenerative changes in the hepatocytes were observed.

In a more recent, but poorly conducted, study, Fischer rats were continuously exposed to trichloroethylene for 12 weeks (Arai et al., 1988). Twelve rats were exposed and four were used as controls. Exposures were initially at 400 ppm (two weeks) rising to 1,500 ppm in the next 2 weeks. Three rats died in the fifth week, so the trichloroethylene concentration was reduced to 600 ppm between weeks 6 and 8. A further rat died in the eighth week and another in the ninth week. The cause of death was unclear. Weight gain was reduced in the decedents, but no histopathological examinations were carried out. From the ninth to the twelfth week the exposure level was 550 ppm. At the end of the exposure period, urine and blood were collected, organs excised and weighed and livers and kidneys were examined histologically. Increased relative liver weights (2.8-fold) were observed and, histologically, centrilobular liver cell enlargement was seen together with clumping of the Kupffer cells and some liver cell necrosis. Blood plasma GPT and alkaline phosphatase activities were increased (approximately 5-fold and 3-fold, respectively). There was no evidence of fatty degeneration, but there were decreases in total plasma cholesterol and triglyceride concentrations (both to around 63% of control values). Plasma albumin and albumin/globulin ratios were slightly increased.

In relation to the kidneys, changes were seen in several parameters including serum urea nitrogen, serum creatinine and urinary amino acids but they were not statistically significant. Urinary glucose excretion was markedly increased, suggesting possible damage to the proximal tubules. Relative kidney weights were increased (42%) but there was no morphological evidence of gross or microscopic damage. Haemoglobin and haematocrit values were both reduced in the exposed rats (to 90% and 80%, respectively, of control values) but in the absence of further investigations the anaemia cannot be categorised.

Overall, little can be concluded from this poor study except that there were indications of liver damage, and possibly also of kidney damage, in rats following continuous exposure to an average of about 800 ppm of trichloroethylene for 12 weeks.

In another study, no biologically significant hepatic effects related to exposure to trichloroethylene were observed in female Wistar rats following exposure to either 500 ppm trichloroethylene alone for 2 hours/day, 5 days/week for 2 months or to trichloroethylene plus drinking water containing 3% ethanol (Danni et al., 1984). Control groups were exposed to water alone or to aqueous ethanol alone. The parameters measured were: body weights and relative liver weights; serum enzymes and bile acids; liver triglycerides; clearance of both bromosulphophthalein (BSP) and sorbitol; hexobarbital sleeping times. Sorbitol clearance (an indicator of "functional liver plasma flow") was reduced to virtually the same extent in animals exposed to trichloroethylene alone (reduced to 60% of control values, $p < 0.001$) as in those co-exposed to alcohol (58.5%, $p < 0.005$) but there was no effect in animals exposed to alcohol alone. BSP clearance (a measure of liver function) was significantly affected (increased) only in

animals co-exposed to alcohol and trichloroethylene (20% increase, $p < 0.05$). None of the other parameters measured showed any significant changes. Little of toxicological significance can be deduced from these observations.

In a limited study to find sensitive markers of liver toxicity, groups of rats were exposed either to 200 ppm of trichloroethylene continuously or to 1,000 ppm for 6 hours per day over 28 days (Wang and Stacey, 1990). Control rats were exposed to air. In this study, only serum bile acid levels were measured. Both cholic acid and taurocholic acid found at significantly higher levels ($p<0.001$) in rats exposed continuously to 200 ppm but not in rats exposed intermittently to 1,000 ppm. The toxicological significance of these changes is unclear.

In a series of briefly reported experiments, Kjellstrand et al. (1981, 1983a, 1983b) used mice, rats and gerbils to investigate the effects of exposure to trichloroethylene (containing 0.01% thymol and 0.03% diisopropylamine as stabilisers). In the first experiment NMRI mice, Sprague-Dawley rats and Mongolian gerbils were exposed continuously to 0 or 150 ppm of trichloroethylene for 30 days. Further investigations were then performed to compare the effects of trichloroethylene on seven strains of mice (wild, C57BL, DBA, B6CBA, A/sn, NZB and NMRI) also exposed continuously to 0 or 150 ppm for 30 days. Finally, concentration-effect relationships were examined in NMRI mice exposed either intermittently or continuously to between 37 and 3,600 ppm of trichloroethylene for 30 or 120 days. At the end of each experiment, liver, kidneys and spleen were taken and weighed.

The first experiment showed that liver weights were increased relative to those of control animals in all three species and that in mice these increases were considerably more marked (60 to 80% increases in mouse liver weights after a 30-day exposure compared with 10 to 20% increases in rats and gerbils).

The results from the second series of experiments showed that the change seen in the liver weights of NMRI mice was not strain specific. Of all the strains tested, NZB mice appeared to be the most sensitive, with liver weight increases twice those recorded for any other strain.

There were no consistent changes in kidney or spleen weights following exposure to trichloroethylene in any of the strains of mice or in rats and gerbils.

When the concentration-effect relationship was examined, it was apparent that liver weights increased in a concentration-related fashion in all groups of NMRI mice (20 animals per group) exposed continuously or intermittently to between 37 and 3,600 ppm of trichloroethylene for 30 days or 120 days. Histopathological examination revealed that the changes in weight were accompanied in both sexes by increases in the size of the hepatocytes and increased vacuolation of the cytoplasm, with some variations in the size and shape of the nuclei. These changes were more marked after 120 days of exposure than the changes seen after 30 days. It is not clear if these changes in the liver represent an adverse effect or whether they are related to adaptive increases for xenobiotic metabolism. Increased kidney weights were apparent in males exposed to 75 ppm or more of trichloroethylene, and in both sexes exposed to 150 ppm or more, for 30 days. Histological examinations were not carried out on kidney. It is probable that kidney weight changes are also adaptive responses for xenobiotic metabolism. Liver and kidney weight changes, and some of the morphological changes in liver, were reversed following a recovery period of 30 days. A “no-effect level” was not established for the hepatic changes, but there were no changes in kidney weights following continuous exposure to 37 ppm of trichloroethylene for 30 days.

Overall, therefore, it seems that the mouse liver is more sensitive to the effects of repeated or continuous exposure to trichloroethylene than rat or gerbil liver, but the changes seen may have been simply adaptive.

An increase in liver mixed function oxidase enzyme activity (cytochrome P-450) was observed in male Wistar rats following exposure to 50 ppm of trichloroethylene (5 hours/day) for 28 days (Norpoth et al., 1974). There were no histological changes in the liver. Changes such as this are to be expected following exposure to many substances as the liver adapts to metabolise the xenobiotic material.

Rabbits (2 animals) survived exposure to 3,000 ppm of trichloroethylene (7 hours/day, 5 days/week) for about 5 weeks (Adams et al., 1951). The only signs of toxicity were CNS disturbance (lack of co-ordination) and increased liver and kidney weights were reportedly observed at necropsy but the data were not given in the report. Reportedly also, histological examination revealed no evidence of liver or kidney damage. Other rabbits, and also guinea pigs, were exposed to 200 or 400 ppm of trichloroethylene (7 hours/day, 5 days/week) for about 8 months. At 400 ppm, body weight gain was reduced in guinea pigs and liver weights were increased in both species at 400 ppm but not at 200 ppm. The no-effect level in this study was 200 ppm. In another study, exposure of a group of eight rabbits to about 2,800 ppm of trichloroethylene (4 hours/day, 5 days/week) for up to 50 days resulted in no deaths. There were no signs of CNS depression and no histopathological changes in heart, adrenals, or spinal cord, but extensive liver damage (degenerative changes in hepatocytes including necrosis) was noted at necropsy (Pennarola et al., 1966). Some changes were also seen in the lung, spleen, brain, and occasionally the kidney. Haematological changes (reduced red and white cell counts and reduced haemoglobin) have also been reported in rabbits (12 animals) exposed to a similar concentration of trichloroethylene for 45 days (Mazza and Brancaccio, 1967).

Guinea pigs survived exposure to up to about 7,450 ppm of trichloroethylene (30 minutes/day) for 10 or 16 weeks (Lande et al., 1939). Deep narcosis was noted within about seven minutes of starting exposure to this concentration.

Trichloroethylene has also been shown to have low toxicity in other studies in guinea pigs and in a range of other species following repeated exposures. On the basis of the results of a range of clinical chemistry, biochemical and histopathological (heart, lung, liver, spleen and kidney) examinations, no toxic effects were reported for guinea-pigs, dogs or squirrel monkeys exposed to about 700 ppm of trichloroethylene (8 hours/day, 5 days/week) for six weeks (Prendergast et al., 1967). Some evidence of liver dysfunction was reportedly observed however in another early, very briefly reported, study in dogs, after exposure to 500 ppm for three weeks or more (Seifter, 1944). The effects were shown to be reversible in animals allowed a recovery period. No toxic effects were noted when guinea pigs, dogs or squirrel monkeys were exposed continuously (24 hours/day) to 35 ppm of trichloroethylene for 90 days (Prendergast et al., 1967).

No signs of toxicity were noted when Rhesus monkeys were exposed to 400 ppm of trichloroethylene (7 hours/day, 5 days/week) for about seven months (Adams et al., 1951). No effects were noted on haematology or on gross or microscopic morphology.

Limited data are available on the cat. From a single study, the toxicity of trichloroethylene appears to be of the same order of magnitude as in other species (Mosinger and Fiorentini, 1954). Signs of effects on the CNS (lack of co-ordination, collapse) were reported when the animals were exposed to about 5,500 ppm (1 to 1½ hours/day) for 4 to 6 months. At necropsy, lesions were noted in the liver (swollen cells and centrilobular fatty lesions), kidney (distended tubules with cells with vacuolated cytoplasm; hyaline and granular casts; occasional infiltrating

foci with necrotic areas), spleen (histiocytic proliferation) and lymph nodes (hypertrophic follicles; large germinal centres with pale, granulated histiocytes).

General toxicity data are also available from long-term cancer bioassays, which are summarised in the carcinogenicity section, below. In one study, female ICR mice and female Sprague-Dawley rats were exposed to trichloroethylene at concentrations of 0, 50, 150 and 450 ppm, 7 hours/day, 5 days/week, for 2 years; no evidence of non-cancer toxicity was observed (Fukuda et al., 1983). In another study, groups of Sprague-Dawley rats, B6C3F1 mice and Swiss mice were exposed to trichloroethylene at concentrations of 0, 100, 300 or 600 ppm, 7 hours/day, 5 days/week for 104 (rats) or 78 (mice) weeks (Maltoni et al., 1986). Evidence of non-cancer was limited to the observation of kidney tubule meganucleocytosis in male rats of the 300 (incidence 20%) and 600 (78%) ppm groups. A NOAEL for kidney toxicity of 100 ppm can be identified from these data.

Two plausible modes of action for trichloroethylene kidney toxicity in the rat, and their possible relevance to humans, are discussed in the Carcinogenicity section (subsection " Possible mechanisms of trichloroethylene toxicity/carcinogenicity"). One mode of action that has been proposed involves metabolism via the glutathione conjugation pathway to form DCVC, which can be activated by renal β–lyase to reactive metabolites. Species differences in the rates of glutathione conjugation and activation of DCVC by β-lyase have been identified, but these differences are not consistent with the known species differences in sensitivity to kidney toxicity. The presence of metabolites from the glutathione pathway have been detected in humans, but it seems that this is quantitatively a very minor pathway in all species, although this is not convincing evidence of a lack of toxicological significance. A second proposed mode of action involves a trichloroethylene induced increased excretion of formic acid, observed in rat studies, possibly resulting from an inhibition of the methionine salvage pathway. Whether this mode of action can operate in humans is not known. Given the uncertainty surrounding the mode of action by which nephrotoxicity occurs in rats and relevance to humans these findings are considered to be of concern for human health.

Pulmonary toxicity

In a recent and well-conducted study, the effects of repeated exposures to trichloroethylene on the lungs of female CD-1 mice were investigated (Odum et al., 1992). Mice were exposed to 450 ppm trichloroethylene (6 hours/day, 5 days/week) for 2 weeks. During this exposure period, groups of mice were sacrificed at intervals to follow the development of lesions in the lungs.

Marked vacuolation of virtually all Clara cells was apparent after the first day of exposure, but after 4 or 5 consecutive exposures the lungs appeared to be morphologically normal. Following the 2-day break from exposure to trichloroethylene, Clara cell lesions were observed again when exposure resumed and, as in the first week of exposure, the lungs appeared normal again by the end of the second week of exposure. Further investigations of the regenerative processes occurring in mouse lungs were not performed, but restoration of the normal proportion of non-ciliated cells is considered to occur both via recovery of less damaged cells and replication of residual non-ciliated cells (see Section 4.1.2.2).

Neurotoxicity

The potential neurotoxicity of trichloroethylene (purity 99.22%, containing butylene oxide stabiliser at 0.69%) was investigated in Fischer 344 rats in a well conducted (in compliance with GLP) 13-week study (The Dow Chemical Company, 1993). Groups of 12 male and 12 female rats were exposed to trichloroethylene vapour at concentrations of 0, 250, 800 and 2,500 ppm,

7 hours/day, 5 days/week. The concentration of trichloroethylene in the exposure chambers was monitored using a spectrophotometric technique throughout the study and found to be always close to the required concentration. Neurotoxicity was evaluated by weekly clinical observation, monthly functional observational battery (FOB), post-exposure testing of the evoked potential of the visual, auditory and somatosensory systems together with a specific investigation into the trigeminal somatosensory system and extensive neurohistopathology.

Lacrimation was observed in a number of females at 2,500 ppm and in occasional females at 800 ppm; this was also observed in a few males at 2,500 ppm. Bodyweight gain was not affected by treatment. The only noteworthy finding in the FOB was the observation of a slightly increased level of activity in trichloroethylene-exposed females at the 13-week assessment. However, a clear dose-related response was not evident, and it is considered most likely that this apparent change was a reflection of background variation. Changes considered to be treatment-related were reported in the results the electrodiagnostic testing of the visual and auditory somatosensory systems. Medium intensity flash evoked potentials recorded from the visual cortex showed a dose-related and statistically significant increase in the amplitude of the midlatency components in both sexes at 800 ppm (25% greater than controls) and 2,500 ppm (32% greater). With respect to the auditory system, deficits in the click and tone-pip auditory brainstem responses were seen in rats of both sexes at 2,500 ppm; the 16 kHz threshold was elevated by about 15 dB, the 30 kHz threshold was elevated by about 8 dB and the 4 and 8 kHz thresholds were elevated by 4-5 dB. No alterations in the evoked potentials were recorded from the cerebellum, somatosensory cortex, caudal nerve action potentials, low and medium intensity flash evoked potentials recorded from the cerebellum, low intensity flash evoked potentials from the visual cortex or in the trigeminal nerve stimulated somatosensory evoked potential. The only treatment-related histopathology change was found in the auditory system; there was focal loss of hair cells in the upper basal turn of the cochlea in animals from the 2,500 ppm group.

A clear NOAEL 250 ppm for neurotoxicity in the rat was identified in this study. At 800 ppm and above there was evidence of mild neurotoxicity, reflected in increases in the midlatency component of the flash evoked potential recorded from the visual cortex (the authors speculated that this change could have been due to the irritant properties of trichloroethylene or a pharmacological effect, but could not rule out neurotoxicity). At 2,500 ppm there was evidence of ototoxicity, observed as elevated auditory brainstem response thresholds and a focal loss of hair cells in the cochlea.

The effects of exposure to trichloroethylene on CNS and autonomic functions have been studied in rats during and after exposure (Arito et al., 1993; 1994). In the first study, rats were exposed to 0, 300, 1,000 or 3,000 ppm for 8 hours per day or 6,000 ppm for 4 hours per day over 3 days. Implanted electrodes were used to make continuous EEG, EMG, and ECG recordings while the rats were allowed to move freely. In the second study, rats were exposed to 0, 50, 100 or 300 ppm trichloroethylene for 8 hours per day over 6 weeks. Again implanted electrodes were used but in the second study, recordings were made over continuous 32-hour periods at the end of the second, fourth and sixth weeks of exposure.

The results showed that, at higher exposure levels (3,000 and 6,000 ppm), trichloroethylene caused incapacitation of postural maintenance and occasional seizures of the hind limbs during exposures associated with abnormal EEG traces. None of these effects persisted for more than one hour post- exposure. Behavioural and EEG abnormalities were not reported at lower exposure levels even following prolonged exposures.

Changes observed at all exposure levels included increases in slow wave sleep accompanied by decreases in wakefulness during exposure. These effects only persisted into the post-exposure

period in rats exposed to 100 ppm or more for 6 weeks or 3,000 ppm or more for 3 days. It was also reported that the circadian heart rate rhythm was disrupted in a dose-related manner. This was particularly noticeable in rats exposed for 6 weeks although the severity and functional significance of this disruption was not clear.

Attempts were made to correlate the behavioural and sleep/wakefulness changes with levels of trichloroethylene and trichloroethanol in the brain in rats exposed to trichloroethylene for 3 days. It was found that a level of 40 μg/g brain tissue gave rise to decreased wakefulness while 160 μg trichloroethylene/g brain tissue gave rise to abnormal EEG traces and effects on postural maintenance. There is some uncertainty about the role that trichloroethanol may have played in inducing the observed changes in sleep/wakefulness.

Although these investigations suggest that trichloroethylene can overtly affect CNS functions at high-dose levels, giving signs of pre-narcosis, at lower dose levels more subtle changes occur (e.g. altered sleep/wakefulness and heart rate rhythms). However, the biological significance, if any, of these subtle changes is unclear.

In an earlier study, groups of 10 male Wistar rats were used to study the effects of trichloroethylene on swimming performance, exploratory behaviour and maze and avoidance learning (Battig and Grandjean, 1963). Rats were exposed to 0 or 400 ppm of trichloroethylene (8 hours/day, 5 days/week) for 44 weeks. Swimming tests were carried out throughout the exposure period and learning and exploratory behaviour tests were administered towards the end of the study. No abnormalities were seen in behaviour during the study and no treatment-related mortality or reductions in bodyweight gain occurred. The results of the behavioural studies indicated an exposure-time related decrease in swimming speed in trichloroethylene-exposed rats but increased exploratory behaviour in the maze. There was no apparent impairment in learning capacity or in the conditioned avoidance response. No significance for human health is attached to the observations in this study.

In a subsequent study, groups of 9 or 10 rats were exposed to 0 or 400 ppm trichloroethylene respectively for 8 hours per day, 5 times per week for 43 weeks to further study the effects of trichloroethylene on exploratory behaviour Battig (1964). The results showed significantly more exploratory behaviour in a maze in rats exposed to trichloroethylene than in control rats but this was mostly related to a loss of open field avoidance behaviour. Normal behaviour was restored after one week free from exposure.

In other early behavioural studies in which rats were used, impairment of avoidance response to unpleasant stimuli was noted in rats repeatedly exposed to 125 to 4,380 ppm of trichloroethylene (4 to 8 hours/day, 5 days/week) for one to forty weeks (Grandjean and Battig, 1964; Goldberg et al., 1964a; 1964b). Little was reported on signs of toxicity: none were observed in rats exposed to 125 ppm (4 hours/day; 5 days/week for 5 weeks) but reductions in avoidance behaviour were reported ($p < 0.01$) after three weeks of exposure at this level. Increased activity (ambulation, preening, rearing) was noted during repeated exposure (6 hours/ day for 5 days) to 200 ppm of trichloroethylene (Savolainen et al., 1977). These results thus suggest that exposure to concentrations of trichloroethylene that produce no overt signs of CNS toxicity may produce subtle effects on the CNS resulting in effects being noted in certain behavioural tests following exposure to trichloroethylene at 125 to 200 ppm and above. However, these studies were not well reported and the small changes observed may have been influenced by the odour of trichloroethylene. Hence, they are considered of little relevance to human health.

There is also evidence that exposure to trichloroethylene can have a transient effect on visual evoked potentials in rabbits. In one study, groups of 4 to 6 New Zealand white rabbits were

exposed (4 hours/day, 4 days/week) for 12 weeks to 350 or 700 ppm of trichloroethylene (Blain et al., 1992). In addition to weekly recordings of visual evoked potentials (VEPs), blood samples were collected to determine the concentration of trichloroethylene and the metabolites trichloroethanol and trichloroacetic acid. Results showed significant ($p < 0.001$) decreases in amplitudes of VEPs at 350 ppm but significant increases ($p < 0.001$) in VEPs at 700 ppm. These changes correlated best with blood trichloroethanol levels. It was reported that VEPs had returned to baseline values within 6 weeks of the end of exposure. No explanation was presented as to why the responses were different at each dose level. The results of this study are therefore considered to be inconclusive.

Behavioural changes assessed as performance in a maze have also been studied in groups of 8 Mongolian gerbils continuously exposed to 0 or 150 ppm of trichloroethylene for 71 or 106 days (Kjellstrand et al., 1981). A further group of gerbils were exposed for 150 days and allowed a 40-day period free from exposure before maze testing. For all tests, control groups were derived from sex matched siblings of the test gerbils. Results immediately at the end of the exposure period showed differences in performance. Trichloroethylene-treated animals made fewer correct choices in the maze after training than controls. Slight performance differences were still apparent at the end of the recovery period although these were not statistically significant.

Morphological changes in the brain of the Mongolian gerbil have been studied in groups of 6 pairs of animals continuously exposed to trichloroethylene at concentrations of 0, 60 or 320 ppm for 3 months followed by 4 months without exposure (Haglid et al., 1981). At the end of the recovery period, brains were removed from animals for extensive histopathological examinations including electrophoresis of brain proteins.

No treatment related changes in body and brain weights were observed. Light microscopy revealed no treatment-related changes in brain tissues. However, following electron microscopy ultrastructural changes were observed in Purkinje and Golgi cells from all areas studied although it was not possible to assess the severity and functional significance of these changes from the information provided. Changes were also apparent in brain proteins (reductions in total soluble proteins and increases in the glial cytoplasmic protein S100) particularly those obtained from the hippocampus, the posterior cerebellar vermis and the brain stem. These changes were thought to represent an increase in glial cytoplasmic volume. In a previous report from this group (Haglid et al., 1980, cited in Haglid et al., 1981), biochemical changes were found in the hippocampus, posterior cerebellar vermis and in the brain stem compatible with astroglial hypertrophy and/or proliferation. Overall, the results of this study suggest that some areas of the brain may be more sensitive to the effects of trichloroethylene exposure, but it is not clear if the changes observed here are adaptive changes brought about by a demand for greater metabolic capacity or represent true adverse changes.

In a subsequent study, this group examined changes in the lipid composition of the cerebral cortex, hippocampus and brain stem of Mongolian gerbils and Sprague-Dawley rats (Kyrklund et al., 1983; 1986). Gerbils were continuously exposed to 0, 50 or 150 ppm trichloroethylene for 12 months and rats were continuously exposed to 0 or 320 ppm for 5, 30 or 90 days. Additional rats from the 90-day exposure group were allowed a 3-, 10- or 30-day recovery period. Studies in rats revealed that changes in brain lipid composition could be detected after only 5 days of exposure. Only slight changes in lipid composition were seen in gerbils at the lower exposure levels used in this study. These observations cannot be interpreted in relation to the possible mechanism of trichloroethylene-induced CNS toxicity.

Overall, giving most weight to the well-conducted 13-week study from The Dow Chemical Company (1993), a NOAEL of 250 ppm can be identified for neurotoxicity for long-term

repeated exposure to trichloroethylene. At lower levels subtle changes have been observed in some studies, but these were of doubtful significance with respect to human health.

Effects on hearing

In the previously summarised 13-week neurotoxicity study, evidence of ototoxicity were reported at 2,500 ppm, observed as elevated auditory brainstem response thresholds and focal loss of hair cells in the cochlea (The Dow Chemical Company, 1993). The NOAEL for this effect was 800 ppm.

The effect of trichloroethylene on the brainstem auditory evoked response amplitude has been studied in Long-Evans and F-344 rats (Rebert et al., 1991). Groups of 10 male Long-Evans rats were exposed to 0, 1,600 or 3,200 ppm of trichloroethylene (99.8% pure) for 12 hours per day, for 12 weeks and groups of 4 or 5 F-344 rats were exposed to 0, 2,000 or 3,200 ppm for 12 hours per day for 3 weeks. Responses to a multisensory test battery designed to investigate brain-evoked potentials for auditory, visual and somatosensory stimulation were evaluated by electrophysiological measurements after 1, 3, 6, 9 and 12 weeks of exposure and 1 and 2 weeks of recovery.

No signs of systemic toxicity were seen. Results of the multisensory test battery examinations showed that for Long Evans rats exposed to 3,200 ppm of trichloroethylene, brainstem auditory-evoked response (BAER) amplitudes were depressed whereas visual and somatosensory potentials remained normal. Effects were apparent after 3 weeks exposure and continued to the end of the study, after 3 weeks recovery. Furthermore, comparisons of BAER amplitudes at different frequencies revealed that the greatest reduction occurred with a 16 kHz stimulus with smaller effects at 8 or 4 kHz. No evidence of adverse effects was seen in this strain at 1,600 ppm. In F-344 rats a dose related reduction in BAER for the same frequency stimuli was seen at both dose levels. These results show that exposure to trichloroethylene can cause hearing loss in both Long Evans and F-344 rats and whereas 1,600 ppm appears to be a NOAEL for the Long Evans strain, a NOAEL cannot be identified for F-344 rats from this study.

In a subsequent study, these researchers evaluated the effects of combined exposures to trichloroethylene and styrene on the auditory system of male Long Evans rats (Rebert et al., 1993). Rats were exposed for 8 hours per day for 5 days to styrene:trichloroethylene mixtures at concentrations of 0:3,000, 250:2,250, 500:1,500, 750:750, or 1,000:0 ppm and hearing loss was studied by recording the brainstem evoked response. Again, decreases in BAER amplitudes were apparent. These decreases correlated to blood levels of total solvent in a linear dose-addition model showing neither synergistic nor antagonistic effects. Co-exposure to trichloroethylene and styrene therefore does not give rise to a greater hearing loss than would be expected from exposure to each solvent alone.

The effects of trichloroethylene on hearing in Long Evans rats have been further characterised (Crofton and Zhao, 1993). Groups of 10 male Long Evans rats exposed (whole body) to 0, 1,000, 2,000 or 4,000 ppm of trichloroethylene for 6 hours/day on 5 consecutive days. Three weeks after the end of the exposure period, rats were tested for auditory thresholds to 4, 8, 16, 24, 32 and 40 kHz. Additional groups of 8 rats exposed to 0 or 4,000 ppm trichloroethylene were used to investigate the time course of effects on hearing. Auditory thresholds to a 16 kHz tone were assessed prior to exposure, one hour following exposure and then at 1, 2, 4, 8 and 12 weeks post-exposure. These additional rats were then evaluated for auditory responses to 0.5, 1, 2, 4, 8, 16, 24, 32 and 40 kHz tones at 14 weeks post-exposure.

There were slight, transient reductions in bodyweight gain in rats exposed to 4,000 ppm of trichloroethylene but bodyweights had recovered by 2 weeks post-exposure. Significant ($p < 0.05$) hearing loss was apparent in rats exposed to 4,000 ppm for 8, 16 and 24 kHz tones. It was found that hearing loss had a rapid onset so that on the 5th day of exposure a 20 dB loss was observed. By 2 weeks post-exposure, a 40 dB loss was recorded and this was still apparent when rats were tested 14 weeks post-exposure. There were no significant differences in hearing between the control animals and those exposed to 1,000 and 2,000 ppm of trichloroethylene.

Hearing loss and reduction of acoustic startle response has also been assessed in groups of 12 Wistar rats exposed to 0, 1,500 or 3,000 ppm trichloroethylene (99.5% pure) for 18 hours per day, 5 days a week for 3 weeks (Jaspers et al., 1993). Auditory thresholds for 5 and 20 kHz tones were measured before exposure and 1, 3 and 6 weeks post-exposure. Hearing thresholds for 5 and 35 kHz tones were also examined at 5 weeks post-exposure.

There was reduced bodyweight gain during exposure in both treatment groups, although by the end of the recovery period, bodyweights were equivalent to control weights. Assessment of auditory thresholds revealed significant ($p < 0.001$) increases for 20 kHz but not 5 or 35 kHz tones in rats at the top dose level (3,000 ppm) only. These changes were apparent in the first week post-exposure and did not show any signs of improvement in subsequent weeks. Furthermore, trichloroethylene-exposed rats failed to show an increase in baseline startle with repeated exposures in comparison to controls. These results are consistent with those obtained by Rebert et al. and Crofton and Zhao, summarised above.

Overall, therefore, these studies on auditory responses to trichloroethylene exposure suggest that repeated exposures of rats to trichloroethylene at concentrations of 2,500 ppm and above may give rise ototoxicity. It is likely that this effect is a specific effect on the hair cells of the organ of Corti. A NOAEL of about 800 ppm of trichloroethylene can be identified for this effect from the studies above.

Immunotoxicity

Aranyi et al. (1986) investigated the effects of 5 consecutive daily 3-hour inhalation exposures in mice on their susceptibility to experimentally induced streptococcus aerosol infection and pulmonary bactericidal activity against inhaled *Klebsiella pneumoniae*. Single exposures were also used- see Acute Toxicity section for study summary. The airborne exposure concentration was 2.5 ppm. There were 30 mice in each group, and five replicate challenges for the streptococcus study and 18 mice in each group for the bactericidal assay. In the treated group, mortality from streptococcal pneumonia was slightly greater than seen in the control groups, but the difference was not statistically significant. Bactericidal activity was not influenced by treatment. Note that in the acute study, effects on mortality were seen at exposure levels of 25 and 50 ppm, but the toxicological significance of these findings is unclear because the assay has not been validation as a toxicity test for chemicals.

Summary of repeated or continuous inhalation toxicity of trichloroethylene in animals

No standard 28-day or 90-day repeated dose general toxicity inhalation studies are available, but an extensive range of other studies has been conducted, including long-term cancer bioassays neurotoxicity tests.

Deaths were reported in one study in rats in which exposure was to 3,000 ppm of trichloroethylene (6 hours/day for 6 months), but in other studies rats and several other species survived exposures between 1,000 ppm and over 7,000 ppm for 90 days. The main toxic effects

which have been observed following repeated inhalation exposure of animals to trichloroethylene are effects on the pulmonary system, liver and kidney, nervous system and on hearing.

Pulmonary effects (vacuolation of Clara cells) were observed in the lungs of mice exposed to 450 ppm of trichloroethylene (6 hours/day, 5 days/week) for 2 weeks. Only this single exposure level was used so a no-effect level is not available. As with similar effects observed following single inhalation exposures of mice, the Clara cells returned to normal during the exposure period (see above).

There are clear effects on the liver (e.g. increased liver weight, increased P450 activities, increased serum enzyme markers of liver dysfunction, fatty infiltration, centrilobular cell enlargement) following repeated or continuous inhalation exposure of rats, mice, rabbits and guinea pigs to trichloroethylene at concentrations of 37 ppm (continuous, 30 days) and above. The mouse is more sensitive than the rat. Many of the reported effects at the lower end of the range of exposure levels used (i.e. below 800 ppm) are reversible and are those to be expected from adaptive changes for xenobiotic metabolism. Taking this into account, a NOAEL of 200 ppm can be derived for liver from the data summarised above.

Some of the effects seen in relation to kidney (e.g. increased weight occurring at 75 ppm) may also be ascribable to adaptive changes for xenobiotic metabolism. Biochemical indications of proximal tubule damage were reported in rats exposed to 800 ppm of trichloroethylene (continuous exposure for 12 weeks) but there were no histological changes in another study in which exposure was to 400 ppm for eight months (7 hours/day, 5 days/week), although kidney weights were increased. In a long-term cancer bioassay, kidney tubule meganucleocytosis was seen at 300 and 600 ppm in male rats, but not female rats of mice of either sex; the NOAEL for kidney toxicity in this study was 100 ppm.

A clear NOAEL 250 ppm for neurotoxicity in the rat was identified in a well-conducted 13-week study. At 800 ppm and above there was evidence of mild neurotoxicity, reflected in increases in the midlatency component of the flash evoked potential recorded from the visual cortex. The results of studies on auditory responses to trichloroethylene exposure suggest that repeated exposure of rats to trichloroethylene at concentrations of 2,500 ppm and above may give rise to ototoxicty. It is likely that this effect is a specific effect on the hair cells of the organ of Corti. A NOAEL of about 800 ppm of trichloroethylene was clearly identified for this effect from the studies summarised above.

Overall, from the available data on the effects of repeated inhalation exposure of animals to trichloroethylene, a NOAEL of 100 ppm can be derived.

Oral

In a study in rats, all the animals died following repeated dosing with 5,600 mg/kg body weight per day with trichloroethylene in corn oil (5 days/week for six weeks) (NCI, 1976). Signs of toxicity noted at 3,000 mg/kg/day and above included loss of weight, hunching, alopecia and laboured breathing. No significant adverse effects were noted on gross examination at autopsy but no histopathology was performed. In mice, all the animals died following repeated administration of 10,000 mg/kg/day in corn oil (5 days/week for six weeks) and no gross lesions were noted at autopsy. No histopathology was performed on either species.

Thirteen-week repeated dose studies have been conducted in F344 rats and B6C3F1 mice to provide data to assist the choice of dose levels for subsequent long-term cancer bioassays (NTP 1990). Groups of 10 animals of each sex were dosed by gavage for five days/week. The animals

were observed daily, subjected to a necropsy at termination and histology was conducted on a wide range of tissues from the high-dose group (rats) or two highest dose groups (mice) and controls. For male rats the dose levels were 0, 125, 250, 500, 1,000 and 2,000 mg/kg/day and for female rats, 0, 6.25, 125, 250, 500 and 1,000 mg/kg/day. The only evidence of toxicity was observed histologically; at the highest dose level, pulmonary vasculitis was found in 6 males and 6 females and minimal/mild cytomegaly and karyomegaly of the renal tubule was found in 8 males and 5 females.

The dose levels for mice of either sex were 0, 375, 750, 1,500, 3,000 and 6,000 mg/kg/day. There were mortalities at 1,500 mg/kg/day and above, with most animals at the highest dose level dying. Male bodyweight gain was reduced at 750 mg/kg/day and above; female bodyweight gain was not affected. Dose related increases in liver weight were apparent for males at 750 mg/kg/day (10% relative to controls) and above and for females at 1,500 mg/kg/day (10% relative to controls) and above. In the liver, centrilobular necrosis was seen in 6 males and one female from the 6,000 mg/kg/day group. Mild or moderate cytomegaly and karyomegaly of the renal tubule was observed in many animals of either sex receiving 3,000 or 6,000 mg/kg/day.

In the long-term (2 year) cancer bioassays, which are summarised in the carcinogenicity section, male and female rats were dosed by gavage, 5 days/week, at levels of 0, 500 or 1,000 mg/kg/day and male and female mice were similarly dosed at 0 or 1,000 mg/kg/day. In rats, the survival of males was statistically significantly reduced in both trichloroethylene treated groups; the numbers surviving to the end of the study were 35/50 (70%), 20/50 (40%) and 16/50 (32%) in the control, low and high-dose groups, respectively. There were adverse effects on bodyweight gain for high-dose males (body weight was 87% of vehicle control value at end of study), low-dose females (88%) and high-dose females (82%). Renal cytomegaly was observed in virtually all trichloroethylene treated animals of both sexes, but not in the controls. In the trichloroethylene treated mice, male survival rate was reduced in comparison with the controls (probability of survival in the treated group was roughly half that of the control group). Also, male bodyweight was reduced in the treated group, to 90% of the vehicle control value. Renal cytomegaly was observed in most treated animals of both sexes, although the lesions were not as severe as those seen in the rats. This study showed that the kidney is a target organ for trichloroethylene in rats and mice of both sexes; NOAELs for long-term administration could not be identified.

Thirteen-week repeated dose studies have also been conducted in ACI, August and Marshall strain rats to provide data to assist the choice of dose levels for subsequent long-term cancer bioassays (NTP, 1988). Groups of 10 animals of each sex were dosed by gavage for five days/week. The animals were observed daily, subjected to a necropsy at termination and histology was conducted on a wide range of tissues from the high-dose groups. The dose levels were 0, 125, 250, 500, 100 and 2,000 mg/kg/day for the males and 0, 62.5, 125, 500 and 1,000 mg/kg/day for females. The only change in survival was seen in high-dose August males, where three males died. Bodyweight gain was reduced among high-dose males in all three strains; reductions of 12-17% at termination, relative to controls, were reported. There were no treatment-related histopathological changes.

In the long-term (2-year) cancer bioassay which is summarised in the carcinogenicity section, rats of the ACI, August, Marshall and Osborne-Mendel strains received oral (gavage) doses of trichloroethylene at levels of 500 or 1,000 mg/kg/day, 5 days/week (NTP, 1988). Survival was significantly reduced, relative to the controls, in ACI males and high-dose females, in high-dose female Osborne-Mendel rats, and in female Marshall rats. Renal cytomegaly was seen in virtually all trichloroethylene treated animals of all four strains. Additionally, toxic nephropathy (distinguishable from age-related nephropathy), characterised by dilated tubules lined by

elongated and flattened epithelial cells, was seen in all treated groups; between 17 and 80% of rats from each group were affected. This study confirmed that the kidney is a target organ for trichloroethylene in rats; again, a NOAEL was not identified.

In another carcinogenicity bioassay, which is also summarised in the carcinogenicity section, groups of 30 rats of each sex received gavage doses at levels of 0, 50, or 250 mg/kg/day, 4 or 5 days/week for 52 weeks and were observed until spontaneous death (Maltoni et al., 1986). Evidence of general toxicity was limited to the observation of kidney tubule meganucleocytosis in 47% of males at 250 mg/kg/day only; females were not affected.

Another study, limited primarily to investigating effects on the liver, revealed no effect on the liver function of rats, as indicated by serum aminotransferase levels, when animals were given 1,000 mg/kg body weight/day of trichloroethylene in corn oil, daily for up to ten consecutive days (Elcombe et al., 1981). However, increases in the relative liver weight and in the hepatic levels of enzymes concerned with the biotransformation of chemicals were seen. Similar effects were observed in mice dosed with 500 mg/kg/day (in corn oil) for ten consecutive days, together with a significant increase in liver non-protein sulphydryl content.

Induction of the mixed function oxidase enzymes was seen in mice dosed with up to 1,600 mg/kg body weight/day of trichloroethylene (in corn oil) for six weeks (Buben and O'Flaherty, 1981) but not in mice treated with 5 mg/kg in the drinking water for up to six months (Barnes et al., 1980) although an increase in liver weight was noted in the latter study. Few data were given in these papers with which to assess the significance of the results.

The biochemical, histological and ultrastructural changes in the livers of male Alderley Park and Osborne-Mendel rats and B6C3F1 and Alderley Park mice following gavage administration of trichloroethylene have been studied to evaluate strain and species differences in hepatic effects (Elcombe et al., 1985). Trichloroethylene (99.9% pure, containing 0.02% triethylamine) was administered in corn oil at doses of 500, 1,000 and 1,500 mg/kg body weight daily for 10 days. Control animals were dosed with corn oil. At sacrifice, livers were removed and the left lobe used for histopathological examination and electron microscopic studies, and the remaining liver was used for biochemical and other studies (DNA content, DNA synthesis, determination of mitotic figures, peroxisomal enzyme activity (i.e. catalase and peroxisomal β-oxidation, examined only in livers from animals treated with 1,000 mg/kg/day).

No significant effects were observed at any dose level on body weight gains, but marked increases were seen in liver weights in mice relative to body weights (up to 175% of control values). Smaller increases, up to 130% of control values, were seen in rats. Hepatic DNA concentration was markedly reduced in mice (down to 66% of the control value) and to a lesser extent in rats (83% of control values). A very marked increase (500% of control values) in DNA synthesis ([^{3}H]thymidine incorporation) was seen in mice but not in rats. The increased DNA synthesis in mouse liver was thought to represent semi-conservative replication of DNA, not repair, since there was a parallel increase in mitotic figures and there were no signs of regenerative hyperplasisa (necrosis). In rats, a ten-fold higher mitotic index was seen in control animals than in control mice and treatment with trichloroethylene resulted in a marked decrease in the frequency of mitotic figures. Thus, the trichloroethylene-induced hepatomegaly in mice was attributed to both hypertrophy and hyperplasia, but only cell enlargement occurred in rat liver. All of the observed changes occurred at all three dose levels and there was little evidence of a dose-response relationship.

In mice, but not in rats, an increase in hepatic peroxisomal enzyme activity was noted. The increase was 8-fold for β-oxidation and 1.5-fold for catalase. Electron microscopy revealed

significant increases in peroxisome volume density (up to 1,110% of control levels) in both strains of mice following treatment with trichloroethylene. Induction of peroxisomes reached a maximum in B6C3F1 mice dosed at 1,000 mg/kg body weight. No significant histopathological signs of hepatotoxicity were seen in rats, and there was no significant increase in peroxisomes.

It is suggested that the differences seen between these two species can explain the species differences in trichloroethylene hepatocarcinogenicity. This is addressed in more detail in the section on mechanisms of toxicity, below.

In another study by the same group, trichloroethylene (> 99.9% pure, containing 0.02% triethylamine) in corn oil was administered by gavage to male Alderley Park mice and rats for 10 consecutive days at dose levels of 50 to 2,000 mg/kg body weight/day (Elcombe, 1985). Further groups of mice and rats were given trichloroacetic acid in corn oil at dose levels of 10 to 200 mg/kg/day for 10 consecutive days. Control animals received corn oil alone. Twenty-four hours after the final dose, the animals were killed and the livers removed. Liver weights were recorded and catalase activity and cyanide insensitive palmitoyl CoA oxidation were measured as markers of peroxisome proliferation. In livers of mice, following oral doses of both substances, dose-dependent increases were seen in cyanide insensitive palmitoyl CoA oxidation, a measure of peroxisomal β-oxidation. No effect was seen on catalase activity following dosing with either substance. In rats, no effects were seen on either of the markers of hepatic peroxisomal proliferation following trichloroethylene administration, but dosing with trichloroacetic acid induced an increase in hepatic peroxisomal β-oxidation. Again, no effect was seen on catalase activity. It is postulated that the species differences in peroxisome proliferation are the results of differences in the rate of formation of trichloroacetic acid from trichloroethylene in mice and rats. This postulate is further related to species differences in the hepatocarcinogenicity of trichloroethylene (see the mechanisms section, below).

In a study designed primarily to investigate the mechanism of rat kidney carcinogenicity, ten male F344 rats were dosed with 2,000 mg/kg bodyweight of trichloroethylene (99.5% pure) in corn oil by gavage once daily for six weeks (Green et al., 1990). Control animals were dosed with corn oil alone. Blood and 24-hour urine samples were taken on days 1, 9, 17 and 42 and livers and kidneys were removed, weighed and examined histopathologically 24 hours after the last dose of trichloroethylene. Plasma levels of alkaline phosphatase (ALP), ALT, urea, creatine, and glucose, urine volume and urinary creatinine, protein, ALP and N-acetyl glucosaminidase (NAG) were determined.

There were no clinical signs of toxicity and body weight gain was reportedly unaffected by the treatment with trichloroethylene. Relative liver and kidney weights were both increased (1.6-fold and 1.3-fold, respectively; both $p < 0.01$). Only the 42-day data from the clinical chemistry tests were presented: plasma ALP levels were slightly increased ($p < 0.05$). Urinary volume and urinary protein, glucose, ALP and NAG were all increased ($p < 0.01$ for all except glucose where $p < 0.05$). Histopathology showed marked hypertrophy in the centrilobular region of the liver but there were no changes in the kidneys.

Urinary NAG is considered a definitive early marker for nephrotoxicity, and the increases observed in this parameter in this study were accompanied by other changes commonly associated with kidney toxicity. Hence, the urinary changes in this study may be interpreted as being indicative of nephrotoxicity following repeated oral dosing with a high level (2,000 mg/kg/day) of trichloroethylene, even though no histopathological changes were seen.

Young adult rats were exposed to trichloroethylene in their drinking water to study the effects on learning and on myelin in the hippocampus (Isaacson et al., 1989). Groups of 6 young (21 days

old) male Sprague Dawley rats were dosed as follows: one group received distilled water for 8 weeks; a second group received trichloroethylene for 4 weeks and distilled water for 4 weeks and a third group received trichloroethylene for 4 weeks, then distilled water for 2 weeks, and then trichloroethylene for a further 2 weeks. The experiment was repeated exactly with a second set of rats. The animals were tested to evaluate their ability to perform spatial navigational tasks and, post mortem, the brains of four animals from each group were examined for changes in hippocampal myelin. The modified Morris swim test was used to assess performance in spatial navigational tasks. Neurobehavioural testing was conducted from the end of each exposure period (i.e. after four weeks and 8 weeks) and continued for fourteen consecutive days each time. In the trichloroethylene-exposed animals, exposure (based on consumption of the 312 mg/ml aqueous solution) was calculated to give an average daily dose of 5.5 mg of trichloroethylene for the first 28-day period and, in the rats receiving a second exposure, the average daily dose was 8.5 mg for 14 days.

The results of the tests of performance in spatial navigational tasks were erratic for the first five or six days of testing but more consistent later (suggestive of a training effect) in the control rats and in those exposed to trichloroethylene for a single 4-week period. In contrast, in the rats exposed to trichloroethylene twice, performance had steadied by day two, and was statistically significantly better from then onwards than that of either of the other groups.

Significant decreases were seen in myelination in one region only of the brains of trichloroethylene-treated rats: in the stratum lacunosum-moleculare (particularly in the regio superior) of the hippocampus, in both dosing regimens. The numbers of myelinated fibres in the affected area of the brain were also significantly reduced in the trichloroethylene-exposed animals. All decreases were, however, less severe in the animals exposed only once than in those exposed twice.

The authors cite several theories to try and explain their observations. One of these is that the facilitated performance (whatever its specific neurological aetiology) may be accounted for by an increase in anxiety or arousal state. However, no description of clinical signs was given. Overall, no significance in relation to human health can be attributed to these results.

In a study of the general toxicity of trichloroethylene in male mice, the animals, in groups of six, were given oral doses of 0, 500, 1,000 or 2,000 mg/kg body weight per day of analytical grade trichloroethylene (in groundnut oil) by gavage on 5 days per week for 4 weeks (Goel et al., 1992). The objectives of the study were to elucidate the biochemical, haematological and pathological responses of target organs with a view to identifying the early indicators for chronic effects.

Clinical signs of toxicity were not reported. There were no changes in body weight gain in the treated animals. Various organs were weighed and examined histologically (liver, kidney, spleen, adrenal, thymus, heart and mesenteric lymph nodes). Significant increases in organ weights were recorded only for liver and kidney and only in the top-dose animals. Histopathologically, there was swelling, vacuolation and degeneration/necrosis of hepatocytes as well as marked proliferation of endothelial cells of the hepatic sinusoids in animals dosed at 1,000 and 2,000 mg/kg/day. In animals dosed at 1,000 mg/kg/day, there was histological evidence of toxic nephrosis but no histological changes were apparent in animals given 500 mg/kg/day of trichloroethylene. This was more pronounced in the top dose animals in which glomerular nephrosis, degeneration/desquamation of tubular epithelium and characteristic amyloid deposition in glomeruli were observed. Biochemical changes were seen in all treated groups, in liver and kidney homogenates, although clear dose-responses were not evident. These changes included significant increases in total protein (liver only) and free sulphydryl levels, increased acid phosphatase and catalase activities and decreased δ-aminolevulinic acid dehydratase

(δ ALAD), interpreted as indicating the sensitivity of liver and kidney as target organs in trichloroethylene toxicity.

Haematological studies showed significantly increased erythrocyte counts and reduced white cell counts, but there were no statistically significant changes in haemoglobin, urea nitrogen, creatinine or uric acid levels in the blood of trichloroethylene-treated mice. There was a parallel, dose-related, increase in cell density and a decrease in δ-ALAD activity (top dose only) in the bone marrow.

It is suggested that the changes observed in this study can be mechanistically related to the effects seen in mice in chronic studies. Hepatic peroxisomal proliferation, amyloid deposition in the kidneys and changes in the haemopoietic system, including decreased δ-ALAD activity can, alongside the observations of other investigators of trichloroethylene-induced toxicity, be linked to the development of the observed chronic effects.

The humoral and cell-mediated immune status of male CD-1 mice exposed, by gavage or via drinking water, to trichloroethylene has been studied (Sanders et al., 1982). This study was a follow up to a similar study in which few effects, other than increased liver and kidney weights, were seen in male and female mice dosed with trichloroethylene via drinking water using the same dosing regimen as in the present study. The gavage dose levels used were 0, 24 or 240 mg/kg body weight, which were given to male mice only, daily for 14 days. In the drinking water study, male and female mice were used. They were exposed for 4 or 6 months to dosing solutions containing 0.1, 1.0, 2.5 or 5 mg/ml of trichloroethylene dissolved in deionised water with 1% emulphor to maintain the trichloroethylene concentration in the water. These concentrations should give doses approximately equivalent to 5 to 250 mg/kg body weight per day. Loss of trichloroethylene from the water was found to be less than 25% of the 1.0, 2.5 and 5.0 mg/ml solutions but up to 45% of the 0.1 mg/ml solution. A range of tests for immune status were conducted (eg IgM response to sheep erythrocytes (sRBC) using the haemolytic plaque assay; plasma antibody titre was assessed using a haemagglutination test; delayed type hypersensitivity was assessed by determining the sensitivity to sheep red blood cells injected into the footpad; bone marrow DNA synthesis was measured *in vitro* by incorporation of [^{125}I]IUdR and macrophage function was assessed by counting peritoneal cells five days after an intraperitoneal injection of 1 ml of 10% Brewer's thioglycollate).

The results from the 14-day gavage study in male mice showed that the humoral immune response to sRBC was not significantly different in trichloroethylene-treated animals from that of control animals, but the cell-mediated immune response was significantly inhibited in a dose-dependent manner

In the 4-month drinking water study, the humoral immune response to sRBC was inhibited only in trichloroethylene-dosed females on the 4th, and not the 5th, day after immunisation. The response was not clearly dose-related and was no longer evident at the end of the 6-month exposure period. The reliability of this apparent effect is doubtful. There were no consistent trichloroethylene-induced effects in males dosed via the drinking water.

The authors themselves say that the results of this study are “unremarkable”, and no attempt was made to interpret the differences observed between the effects in gavage-dosed and drinking-water-dosed male mice. Overall, this study contributes little to the understanding of the toxicity of trichloroethylene.

The trichloroethylene-induced autoimmune response in female MRL +/+ mice (a strain prone to a lupus-like autoimmune disease) has been investigated in a study that used the intraperitoneal

route (Khan et al., 1995). Groups of four or five 4-week old mice received injections of either 10 mmol/kg (1,300 mg/kg) trichloroethylene or dichloroacetyl chloride (DCAC, a metabolite of trichloroethylene) every 4th day for 6 weeks. A similar control group received injections of corn oil. Animals were killed 24 hours after the final injection and sera and major tissues were sampled. Spleen weights were increased by about 36% in both the trichloroethylene and DCAC treated groups. Serum IgG levels were increased by 45% and 322% in the trichloroethylene and DCAC groups, respectively. The presence of certain autoimmune antibodies were detected in the following numbers of control, trichloroethylene and DCAC treated animals, respectively: anti-nuclear antibodies, 0/4, 4/4, 3/5; anti-ssDNA antibodies 0/4, 2/4, 5/5; anti-cardiolipin antibodies 0/4, 0/4, 5/5. Although these results may raise some concerns about potential immunotoxicity, no firm conclusions can be drawn from this study in relation to the hazardous properties of trichloroethylene in the context of a risk characterisation for human health because the study design and animal model have not been validated for toxicological testing. Furthermore, the small numbers of animals used and difficulties in extrapolating the results from intraperitoneal administration to more relevant routes makes it difficult to draw any firm conclusions.

Wright et al. (1991) investigated the immunotoxic effects of trichloroethylene on spleen and hepatic derived lymphoid cell populations from Sprague-Dawley and B6C3F1 mice, following short-term *in vivo* and *in vitro* exposure. Rats receiving three consecutive daily intraperitoneal doses of 5 mmol/kg (650 mg/kg) trichloroethylene showed a reduction in spleen weight and spleen cell count; similar effects were seen in mice at 10 mmol/kg (1,300 mg/kg, the only dose level tested). No effects on the spleen were seen in rats at 0.5 mmol/kg (65 mg/kg). In *ex vivo* studies, single does of trichloroethylene produced a reduction in natural killer activity in hepatic but not splenic effector cells. Rat hepatic natural cytotoxic cell activity and natural P815 killer activity was also inhibited by trichloroethylene. In a further *in vitro* study, available only as an abstract, Wright et al. (1994) demonstrated the inhibition by trichloroethylene of human hepatic natural killer, natural cytotoxic and natural P815 killer cell activities. As for the Khan et al. study above, no firm conclusions can be drawn from the Wright studies in relation to the hazard properties of trichloroethylene in the context of a risk characterisation for human health because the this study design has not been validated for toxicological testing.

Dermal

There are no data available on the effects of repeated exposure of animals to trichloroethylene by the dermal route.

Summary of repeated dose toxicity in animals

Although no standard 28-day or 90-day repeated dose inhalation or oral studies with a full range of observations are available, an extensive range of studies has been conducted. The main toxic effects which have been observed following repeated inhalation exposure of animals to trichloroethylene are effects on the liver, kidney, pulmonary and nervous systems and on hearing. Liver and kidney toxicity have also been reported following oral administration.

Pulmonary effects (vacuolation of Clara cells) were observed in the lungs of mice exposed to 450 ppm of trichloroethylene (6 hours/day, 5 days/week) for 2 weeks, but a no-effect level is not available.

There are clear effects on the liver (e.g. increased liver weight, increased P450 activities, increased serum enzyme markers of liver dysfunction, fatty infiltration, centrilobular cell enlargement and, at very high doses, centrilobular necrosis) following repeated inhalation or oral exposure of animals to trichloroethylene. The mouse is more sensitive than the rat and, in oral

studies, peroxisome proliferation has been shown to occur in the livers of trichloroethylene-treated mice, but not in those of rats. Many of the reported effects at the lower end of the range of exposure levels used in inhalation studies (i.e. below 800 ppm) are reversible and are those to be expected from adaptive changes for xenobiotic metabolism. Taking this into account, a NOAEL of 200 ppm for inhalation exposure can be derived for liver from the data summarised above. No evidence of liver toxicity was seen in oral long-term cancer bioassays in rats and mice, conducted at dose levels of 500 mg/kg/day and above; a NOAEL of 500 mg/kg/day is therefore suggested for liver toxicity.

Regarding the kidney, tubule meganucleocytosis was seen at 300 and 600 ppm in male rats, but not in female rats or mice of either sex in a long-term cancer bioassay using the inhalation route. A NOAEL of 100 ppm was identified from this study. By the oral route, trichloroethylene elicited kidney toxicity in rat and mouse cancer bioassays at dose levels of 250 mg/kg/day and above; a NOAEL of 50 mg/kg/day can be identified. The kidney toxicity was characterised by tubular cytomegaly and dilatation. There are two biologically plausible modes of action for trichloroethylene kidney toxicity in rodents. One involves the formation of reactive intermediates in the kidney by β-lyase activity and the other involves the increased excretion of formic acid. However, neither mode of action can be considered proven and, furthermore, there is uncertainty as to whether these could operate in humans.

A clear NOAEL 250 ppm for neurotoxicity in the rat was identified in a well-conducted 13-week study. At 800 ppm and above there was evidence of mild neurotoxicity, reflected in increases in the midlatency component of the flash evoked potential recorded from the visual cortex. The results of studies on auditory responses to trichloroethylene exposure suggest that repeated exposure of rats to trichloroethylene at concentrations of 2,500 ppm and above may give rise to ototoxicty. It is likely that this effect is a specific effect on the hair cells of the organ of Corti. A NOAEL of about 800 ppm of trichloroethylene was clearly identified for this effect from the studies summarised above.

The results of studies on auditory responses to trichloroethylene exposure suggest that short-term inhalation exposures of rats to trichloroethylene at high concentrations (3,000 ppm and above) may give rise to persistent mid-frequency hearing loss. It is likely that this effect is a specific effect on the hair cells of the organ of Corti. A NOAEL of about 2,000 ppm of trichloroethylene can be identified for this effect.

Overall, kidney toxicity appears to be the most sensitive endpoint for both long-term repeated inhalation and oral exposure. For inhalation exposure, the NOAEL is 100 ppm. For oral exposure a NOAEL of 50 mg/kg/day be identified.

4.1.2.6.2 Studies in humans

This section incorporates and updates the study assessments presented in an earlier HSE review (HSE, 1982).

Inhalation

All studies conducted in occupationally exposed populations have been considered under the heading of inhalation exposure. However, it is recognised that for many individuals some trichloroethylene exposure is likely to have taken place via the dermal route.

Studies of general health and symptoms

- Studies in volunteers

The effects of repeated exposure to trichloroethylene over a four-week period have been investigated in a small number of healthy male volunteers aged from 19 to 46 years (Stewart et al., 1974a). They were exposed to 20 ppm of trichloroethylene for the first week, 100 ppm for the second and third weeks (fluctuating between 50 and 200 ppm in the third week) and 200 ppm for the fourth week, all exposures being for five days a week. The men were divided into three groups of 3-4 men, who were exposed for different time periods each day (1, 3 or 7.5 hours). Each subject was given a full medical examination prior to the start of the study, and was subjected to close medical surveillance throughout the exposure period. Pulmonary function tests were carried out prior to and at the end of the exposure period. Blood was taken for haematology and clinical chemistry three times each week. Electroencephalograph (EEG) tracings were recorded at approximately hourly intervals (pre- and post-exercise) during each exposure period. In addition, each subject performed a number of behavioural tests twice weekly during the exposure period and electroencephalograph recordings were carried out in the subgroup exposed for 7.5 hours a day.

No symptoms of toxicity were observed which could be related to trichloroethylene exposure. No effects were reported on the pulmonary function tests (mean midmaximal flow rates, mean forced vital capacity, and mean peak flow rates), the results obtained at the end of the exposure period being comparable to the pre-exposure values. However the actual results obtained were not given. The only changes noted in the EEG were at 200 ppm; these were described as "minimal" and of no serious consequence.

No impairment was noted in performance of a range of behavioural tests to measure cognitive function (arithmetical test, 90-minute alertness test, response to light and sound stimuli, the Flannagan co-ordination test, a number of choice inspection tests, and a time estimation test). Again, the actual results obtained were not given, and an assessment of the significance of this study is not possible.

As part of this study, the effect of administration of alcohol was investigated during the latter part of the experiment, when the subjects were exposed to 200 ppm of trichloroethylene (Stewart et al., 1974a; Stewart et al., 1974b). Administration of relatively small amounts of alcohol (below 0.5 ml/kg either as beer or brandy) was shown to produce a marked but transient vasodilation of the superficial skin vessels ("degreasers' flush"). The onset of the effect was detected about 20 minutes after drinking the alcohol, the maximum response being obtained about 30 minutes later, and the reaction fading completely after a further 60 minutes. The vasodilation was localised to the superficial vessels in the skin of the face, shoulders and trunk and was not accompanied by any other observable physiological response. The reaction could be demonstrated 72 hours after exposure to the trichloroethylene ceased. In addition, it was known to have occurred in one individual on consuming alcohol six weeks after the last exposure to trichloroethylene. Repeated exposure to trichloroethylene was believed necessary before the alcohol-induced vasodilation could occur. It was known that, contrary to instructions, several of the volunteers consumed alcohol in the evenings; the characteristic "flush" was only noted in some of these during the third week of exposure. No cases occurred earlier.

- Studies in occupationally exposed groups

Numerous health surveys have been conducted in trichloroethylene exposed workers. Unfortunately, generally very little information is available on exposure levels and few studies have included an unexposed control group. Consequently it is difficult to assess the extent to

which any observed health effects or symptoms were be related to trichloroethylene exposure. A summary of the studies to investigate the general signs and symptoms of toxicity in workers exposed to trichloroethylene is given below.

The relationship between exposure to trichloroethylene and liver and kidney toxicity has been examined in metal degreasers (Rasmussen et al., 1993a). A cross sectional study of 240 degreasers was conducted in Denmark. Of these, 99 had used trichloroethylene or CFC 113 for degreasing, and agreed to participate in the study. Their age range was 19 to 68 years (mean 39.4). There was no external control group, but as the period of employment in full time degreasing ranged from 1 month to 36 years, an internal reference group could be established, judged to be suitable for dose(exposure)-response assessment. Each worker underwent a clinical examination at hospital, which included a medical interview and the obtaining of a detailed occupational history with information on the types of solvents used and general occupational hygiene conditions. Neurological and neuropsychological examinations were made, and blood and urine samples were taken for a battery of laboratory tests (serum levels of aspartate aminotransferase, γ–glutamyltransferase (γ-GT), alkaline phosphatase, bilirubin and protein, plasma prothrombin and urinary *N*-acetyl-β–glucosaminidase (NAG).

Based on the total number of exposure hours, four separate groups, of very similar sizes, were identified: group I (reference group) with less than 1 year of full time exposure; group II, 1 to 2.8 years; group III, 2.9 to 6.7 years and group IV 6.8 to 35.6 years. The predominant exposure for 70 of the workers was to trichloroethylene (mean exposure time estimated to be 7.1 years, with exposure for 25 hours/week). “Peak exposures” were defined as exposure for more than 30 hours/week in one year.

Present or recent exposure to trichloroethylene was quantified by reference to the concentrations of trichloroethylene metabolites in blood and urine. In the highest exposure group, the mean urinary trichloroacetic acid concentration was 7.7 mg/l (maximum = 26.1 mg/l). However, historical exposure data, reviewing all measurements of trichloroacetic acid in urine (it is presumed from workers exposed to trichloroethylene, though this is not stated in the report) notified to the Danish Labour Inspection Service during the period 1947 to 1987 indicate a fairly constant exposure level corresponding to a urinary trichloroacetic acid concentration of 40 to 60 mg/l from the mid 1950s to the mid 1970s, which is a time period relevant to this study population (probably indicating atmospheric exposure levels of less than 50 ppm).

None of the study population had any present or previous clinical liver or kidney disease and none was taking known hepato- or nephro-toxic drugs. Age and alcohol abuse (defined as weekly consumption of more than 21 drinks; or periods with treatment for alcohol abuse, which was the case for five persons) were the most relevant confounding factors.

The results showed statistically significant exposure-response relationships for elevated concentrations of serum γ-GT and urinary NAG. However, when age and alcohol abuse were also taken into account this relationship was not statistically significant. For the other parameters, there were no differences between the groups. Overall, this study does not provided any clear evidence trichloroethylene-related impairment of liver or kidney function. The workers in this study were also investigated for neurotoxicity; the results of this investigation are summarised below (Rasmussen et al., 1993b).

The results of a health survey of 50 workers, from 24 different workshops using trichloroethylene degreasing tanks in Switzerland were reported in 1955 (Grandjean et al., 1955). Analysis of the atmospheric trichloroethylene levels at the time of the study revealed that in most cases the concentration near the degreasing bath was in the range 20-40 ppm. However, the

values obtained showed great variation between sites, and also at different times of the day at the same site; the range recorded was 1 to 335 ppm. The average age of the workers was 43 years and the mean length of employment was 3.25 years (range 1 month to 15 years). Each was subjected to an extensive medical examination including physical examination, neurological examination of papillary reflexes, cutaneous and deep sensitivity, cutaneous and proprioceptive reflexes and also hearing and sight. In addition, note was taken of any symptoms experienced during work. The individuals were also subjected to a clinical psychiatric examination. There was no control group included for comparison.

No significant abnormalities were noted in haematology or in blood biochemistry of any of the workers; there was no indication, from serum protein, bilirubin or alkaline phosphatase levels of any marked hepatotoxicity, although serum alkaline phosphatase levels were slightly elevated in four subjects. CNS-related symptoms were reported in most of the workers. These included vertigo (50%), fatigue (44%), headaches (32%), paraesthesia (20%), emotional lability (20%) and memory loss (15%); additionally, intolerance to alcohol (36%) was reported. Neurological examination revealed that some evidence of adverse effects was apparent in 28% of the workers. These included tremors, ataxia, visual disturbances, nystagmus and reduction in cutaneous sensitivity. Disorders suggesting effects on the autonomic nervous system were noted in 36%; these included excessive perspiration, disorders of the circulation and heart rhythm and of the gastrointestinal tract. Examination of the mental state of the subjects indicated some slowing of the mental processes in 40%, reduction in memory in 38% and emotional instability in 26%. No further details of these effects were given, apart from the statement that they occurred with higher frequency in those exposed to trichloroethylene at concentrations above 40 ppm (mean level 85 ppm).

Thus, in this study a relatively high incidence of neurological symptoms (relating to both the CNS and peripheral nervous system) together with psychological disturbances were noted in the trichloroethylene exposed workers. However, since an unexposed control group was not available for comparison, no conclusions can be drawn from this study.

A high incidence of CNS-related symptoms, together with signs of irritant effects on the mucous membranes, were noted in an investigation of 75 people occupationally exposed to trichloroethylene, either at dry cleaning establishments (12 subjects) or through the use of trichloroethylene in industry as a degreasing agent (55 subjects) (Bardodej and Vyskocil, 1956). Unfortunately, again no controls were included for comparison. Atmospheric trichloroethylene levels in the dry cleaning establishment were estimated to be in the range 30-632 ppm and those in the degreasing workshops 5-154 ppm. CNS symptoms were widespread in both groups; the most common effects were headache, sleepiness, feelings of inebriation, nausea and tinnitus. Signs of local irritation to the eyes (lachrymation), skin (reddening) and respiratory tract were also common in both groups. When the workers were subdivided into four groups, based on their duration of exposure (below 1 year, 1-2 years, 2-9 years and over 10 years) the following effects were shown to be related to length of exposure: irritancy to skin and eyes, sleep disturbances, giddiness, tremors, intolerance to alcohol, “severe neuraesthenia syndrome with anxiety state” and bradycardia. Again, because of the absence of a control group, no conclusions can be drawn from this study.

Symptoms of CNS disturbances were commonly reported in a detailed study of 104 workers (87 men, 17 women) exposed to trichloroethylene in Sweden (Anderson, 1957). The subjects worked mainly in factories using trichloroethylene for degreasing purposes, but some were from dry cleaning establishments and the rubber industry. At the start of the study, about half of these workers had been occupationally exposed to trichloroethylene for two years or more. Few data were available on the atmospheric levels of trichloroethylene to which the workers were

exposed, but urinary trichloroacetic acid levels were measured in most cases. Again, no control group was included.

Symptoms of CNS effects were noted in about two thirds of the subjects. These included headache, dizziness, vertigo, fatigue, nausea, vomiting, tremors, sleepiness and feelings of inebriation. The incidence showed some correlation with increasing urinary trichloroacetic acid levels, with most of the individuals who had urinary levels of above 75 mg/l showing some symptoms. Mild symptoms were, however, also noted in the group with the lowest urinary trichloroacetic acid levels. The relevance of these results to exposure to trichloroethylene is not clear, as it was stated that 35 of the workers had personal social problems which probably affected these findings.

There was very little evidence of any effect on the cranial or peripheral nervous system of these workers. This consisted of an isolated case of transient numbness in the area covered by the fifth cranial nerve, and paraesthesia and tremors of the fingers in a few instances. The possibility of exposure to vibration was not mentioned in the report. No abnormalities were noted in haematology or clinical chemistry, and liver function tests performed on a few individuals indicated no adverse effects. In a follow-up study on a number of workers, it was shown that all symptoms disappeared within 4-5 months of cessation of exposure to trichloroethylene. As with the previous studies, no conclusions can be drawn from this study because of the lack of a control group.

Clinical findings have been reported in a small group (8) of male Japanese workers following an outbreak of symptoms of narcosis and general malaise, shortly after the start of the use of trichloroethylene as a solvent for degreasing purposes (Nomura, 1962). An open tank heated to 50^{o}C was used, with no ventilation. Trichloroethylene levels of 230 to 380 ppm were detected in the breathing zone of workers using the tank; a single measurement in the centre of the room gave a value of 115 ppm (although it seems likely, considering the description of the process, that exposures could have been much higher than reported).

The health survey was carried out 13 days after the start of trichloroethylene usage. During the first week, most of the men missed one or more days work due to severe fatigue; other effects also noted included muscular pains, nausea and vomiting. Blood tests showed no significant haematological effects, but an increase in plasma gamma-globulin and a decrease in albumin was noted. Abnormal values in the cephalin cholesterol flocculation test were noted in most (75%) of the workers. An increase in urinary albumin and urobilinogen, but not urobilin was noted. These tests were repeated ten days later, during which time the workers had not been exposed to trichloroethylene. No abnormalities were detected at that time.

These results suggest that workers exposed to levels of trichloroethylene sufficient to produce marked symptoms of toxicity showed some evidence of liver damage. However, because of the lack of a control group, small group size and uncertainties regarding the exposure assessment, no firm conclusions can be drawn from this study.

The results of a health survey at another Japanese machine plant have been reported (Takamatsu, 1962). All 50 employees working in the degreasing area and the adjacent room were included. Most had been employed for 2.5 years at the time of the initial survey at the plant.

Atmospheric trichloroethylene levels (the duration of sampling was not reported for any of the determinations) at that time in the degreasing room were in the range 100-600 ppm, the highest concentrations being obtained in air samples taken from near the floor. The concentrations in the adjacent room were usually between 50 and 100 ppm. It is probable that these measurements, taken from static sampling, were not representative of personal exposure.

Most of the workers had symptoms of CNS toxicity (vertigo, fatigue, headaches, sleeplessness). Many had slight to moderate visual disturbances, and 15% had experienced double vision. No significant haematological abnormalities were noted, but blood biochemistry revealed an increase in serum gamma-globulin and a decrease in serum albumin levels. Urine analysis showed the presence of albumin and urobilinogen in 30% and 36% of the workers, respectively.

A second survey was carried out at the same factory ten months later. The men were divided into three groups, on the basis of their exposure to trichloroethylene. These consisted of eight workers from the degreasing room, where average trichloroethylene concentrations were in the range 150-250 ppm, 14 workers from that part of the assembly room adjacent to the degreasing area, where average concentrations were 50-100 ppm, and 16 workers from the other end of the assembly room, where levels were below 50 ppm. The average urinary trichloroacetic acid levels at the end of the work week were 311 $\pm$ 119 mg/l, 141 $\pm$ 53 mg/l and 50 $\pm$ 24 mg/l in the three groups, respectively, which are consistent with the reported atmospheric levels.

The following symptoms were noted by more than 50% of the workers from the high exposure group: headache, dizziness, giddiness, feelings of drunkenness, flushing of the face, burning in the throat, skin effects and fatigue. Symptoms experienced by more than 50% of the group exposed to intermediate trichloroethylene levels were fewer than in the high exposure group. They consisted of headache, burning sensation in the eyes, flushing of the face and fatigue. Of the workers exposed to below 50 ppm of trichloroethylene, the frequency of symptoms was unclear although it was suggested that some workers were affected.

No effect on haematological parameters was noted in any group, but a marked decrease in serum albumin, and an increase in serum gamma-globulin levels were noted in the group exposed to the highest trichloroethylene concentrations. Slight effects were noted in the intermediate group, but none in those exposed to the lowest trichloroethylene levels.

Thus, in this study, CNS symptoms, together with alterations in blood albumin and gamma-globulin levels, were noted in workers exposed to trichloroethylene levels in the range 150 to 250 ppm. Similar but less marked effects were noted in the workers exposed to 50 to 100 ppm of trichloroethylene, with possible excursions to higher levels. Blood parameters were within the normal range for the group exposed to less than 50 ppm. However, the absence of a control group and uncertainty regarding the frequency of symptoms in the workers exposed to lower levels of trichloroethylene make it difficult to draw firm conclusions, particularly with respect to CNS symptoms, from this study.

The results of a health survey on a group of 70 young workers at a Romanian semi-conductor plant have been reported (Lilis et al., 1969). These were mainly women (about 75%) and mostly below 30 years of age. Most had been employed at the plant for less than two years, the maximum duration of employment being six years. A control group was not available for comparison. Only very limited data were available on the atmospheric trichloroethylene concentrations. These were stated to have exceeded the national MAC value of 50 mg/m^3 (9 ppm) in 40% of determinations and were above 200 mg/m^3 (37 ppm) in 12% of the determinations. No details were given regarding the method of analysis.

Pre-narcotic symptoms were reported by most of the workers during the work-shift. These occurred nearly every day in about one-third of the individuals. Symptoms included, in decreasing order of incidence: dizziness (88%), headache (74%), nausea (43%), euphoria (31%), sleepiness at end of shift (29%), palpitations (29%) and visual disturbances (21%). In nine cases the workers had marked episodes of inebriation, and had to go outdoors. In one case loss of consciousness occurred for a short time. Other symptoms, stated to appear insidiously after

exposures for between several months and two years, included irritability (56%), loss of appetite (50%), excessive sweating (39%) and alcohol intolerance (21%). Physical examination of the workers revealed few significant abnormalities, apart from some neurological disturbances: tremor was present in 31%, hyperactive tendon reflexes in 34%, and nystagmus in 7%. No other effects were noted.

Thus, in this study, a high incidence of CNS disturbances, together with some effects on the peripheral nerves (reduced tendon reflexes) were noted in workers exposed to trichloroethylene levels claimed only to exceed 37 ppm occasionally. However many workers experienced periods of inebriation whilst at work, and one became unconscious. The atmospheric trichloroethylene levels were therefore likely to have been much higher on occasions than those reported. Because of this uncertainty, and the lack of a control group for comparison, no conclusions can be drawn from this study.

Widespread occurrence of similar signs and symptoms were reported in a survey of about 130 workers from several factories in the UK using trichloroethylene (Smith, 1970). These included, in decreasing order of incidence, fatigue (75%), dizziness (56%), gastrointestinal disturbances (25%), headache (18%) and effects on the autonomic nervous system (8%). No information was given on the atmospheric levels at the factory and no control group was included for comparative purposes, but an increased incidence of symptoms was shown with increasing urinary trichloroacetic acid levels. It was stated that about 61% of the workers had urinary levels of trichloroacetic acid below 20 mg/l, 21% had levels of 20-60 mg/l and the remaining 18% had levels above 60 mg/l. The average number of symptoms recorded in individuals from each group was 1.3, 1.8 and 2.7, respectively. Unfortunately, no firm conclusions can be drawn from this study because of the absence of a control group.

Involvement of both the CNS and peripheral nervous system was noted during medical examination of 50 Polish workers (28 male and 22 female) who complained of symptoms of trichloroethylene toxicity (Szulc-Kuberska, 1972). The individuals were from various small factories and dry cleaning establishments. The age range of the subjects was 21 to 55 years, and they had been occupationally exposed for between 1 and 23 years. No data were available on the exposure levels and no control group was included for comparative purposes.

The most frequent symptoms were somnolence (50%), loss of appetite and nausea (20%), intolerance to alcohol (20% incidence, manifest by reddening of cheeks and skin – "trichloroethylene flush") and headaches (18%). Neurological examination revealed a number of apparent functional deficits in cranial and peripheral nerves. Impairment of sensation in the face was noted in six (12%), and weakness of the optical reflex in four (8%). Paraesthesia was noted in seven (14%) and sensory disorders, in the wrists and forearm, in nine individuals (18%).

This study revealed a relatively high incidence of symptoms of CNS depression and inhibition of sensory nerves was noted in workers exposed to trichloroethylene. However, again, it is difficult to draw any firm conclusions because no information was available on the atmospheric levels of trichloroethylene and the lack of a control group for comparison.

The health status of 140 women workers at three Polish workshops using trichloroethylene has been reported (Zielinski, 1973). The average age of the women was 37 years, and the average length of exposure 9.2 years. The results were compared with those obtained in a control group of 44 women stated to be of comparable ages and training, but who were not exposed to trichloroethylene; no further details of this group were given. The mean atmospheric trichloroethylene level in each of the three workshops was stated to be 37 ppm but no

information was given on the concentration range or the variability between sites. No details of the analytical methods used nor the frequency of monitoring were given.

A much higher incidence of the following symptoms was noted in the women exposed to trichloroethylene: drowsiness, general malaise, intolerance to alcohol, reduced appetite and muscle pains. Slight differences were also noted in the haematology findings between the groups (reduced haemoglobin levels, red blood cell and lymphocyte count, and increased monocyte count in the exposed workers). Insufficient data were given for a full assessment of these haematology results, but they appear to be of little significance. No significant difference was noted in serum proteins (including electrophoretic pattern) or bilirubin levels between the exposed and control groups. Sickness records revealed an increased number of accidents at work, which the authors suggested may have been due to trichloroethylene exposure, but no evidence was given to support this.

These results indicate a high incidence of symptoms of CNS depression in women workers exposed to trichloroethylene. There was no significant effect noted on haematology, nor any indication of liver damage from blood biochemistry. It was claimed that the mean atmospheric trichloroethylene levels were 200 mg/m^3 (37 ppm) but no information was given as to the range, or the variability at the three different sites, nor on the method or frequency of monitoring. Because of uncertainties relating to the exposure assessment, no firm conclusions can be drawn from this study.

The results of a clinical examination of 30 male workers from an Egyptian printing factory indicated a widespread incidence of subjective CNS symptoms and irritant effects (El Ghawabi et al., 1973). Trichloroethylene was used at the factory for cleaning the plates used in rotogravure printing. Mean atmospheric trichloroethylene levels, described as being sampled at the breathing zone, were 41 to 163 ppm (range 38-172 ppm) at different parts of the plant; it is not clear if these values represent time weighted averages. Most of the workers had been employed for more than three years, and all but two were below 50 years of age. The results obtained were compared to those found in a group of 30 workers, of comparable age and social class; 20 of these had no occupational exposure to chemical substances whilst ten were also workers from the printing industry but with no occupational exposure to trichloroethylene.

Signs and symptoms noted in the trichloroethylene exposed workers included, in decreasing order of incidence, headache (87%), dizziness (67%), sleepiness (53%) nausea and vomiting (47%) lachrymation (40%), reduced libido (33%), skin rashes and itching (30%) and fatigue (30%). In the control group the only symptoms with an incidence of 10% or more were headaches (30%) and diminished libido (10%). Haematological and liver function tests were also conducted; the results for the control and treated groups were similar.

Thus, in this study an increased incidence of subjective CNS symptoms together with signs of irritancy to the skin and eyes, was seen in workers exposed to mean trichloroethylene levels of up to about 160 ppm.

Similar symptoms were recorded in a health survey of a small group of workers at a tannery where trichloroethylene was used for degreasing purposes (De Rosa et al., 1971). Twenty-four out of a total workforce of 30 men were examined. There was no control group. The average age of the men was 40.5 years, and the mean length of exposure to trichloroethylene was 16.5 months. No information was given on the trichloroethylene levels at the plant, but ventilation was known to be inadequate. Urinary trichloroacetic acid levels 24 hours post-shift were mainly in the range 50 to 100 mg/l.

A high incidence of symptoms, mainly relating to CNS disturbances or to irritancy, was observed in the trichloroethylene workers. These included, in decreasing order of incidence, vertigo (79%), irritability (75%), drowsiness (67%), weakness (67%), dyspeptic disorders (62%), headache (54%), eye irritation (46%), pruritis (37%), smarting in the throat (37%), sweating (29%) and alcohol intolerance (21%). Clinical examination indicated hepatomegaly in five workers (21%) and hyperbilirubinuria was noted in nine (29%). However no effects were noted on serum aminotransferase levels, nor on total serum protein or cholesterol levels. No effect on renal function was noted in any of the workers, nor was there any evidence of peripheral neurotoxicity.

The value of this study is severely limited by the lack of any details regarding atmospheric trichloroethylene levels, and the failure to compare the results obtained with a matched control group. There was evidence of liver disease in some individuals, but insufficient data are given to allow any conclusions to drawn with respect to the relationship between these changes and exposure to trichloroethylene.

Another group of investigators has reported increased serum beta- and gamma-globulins and some abnormalities in the cephalin flocculation test in workers regularly exposed to trichloroethylene (Guyotjeannin and Van Steenkiste, 1958). No details were given on environmental trichloroethylene levels, nor whether any symptoms were noted in these workers and there was no control group. No significance can be attributed to these findings.

Some evidence of liver effects was also noted in a group of 12 women workers from an Italian factory where trichloroethylene was used (Capellini and Grisler, 1958). A control group was not included. All the women examined had been employed at the factory for 2-3 years and had experienced symptoms of CNS disturbances. The trichloroethylene was used as an adhesive to join rubber strips; it was spread over one side of the strip, and allowed to evaporate partially, before joining the strips. No information was given regarding the atmospheric trichloroethylene levels, although urinary trichloroacetic acid concentration was measured; values ranged from 44 to 80 mg/l.

Symptoms in all the workers included headaches, dizziness, and feeling of elation; some had dyspepsia. Physical examination revealed slight enlargement of the liver in six of the subjects, the liver being painful on palpation. Serum gamma-globulin levels were raised in these six individuals, and increased serum bilirubin levels were noted in two. No effects were however observed on serum total protein, albumin, cholesterol (total and esterified) and alkaline phosphatase.

The value of this study is limited by the very small numbers of subjects, the lack of information regarding the atmospheric trichloroethylene levels and any concomitant exposures (e.g. to alcohol), and the failure to use a control group.

Abnormalities in liver function were reported in an investigation of 22 workers (15 male, 7 female) exposed to trichloroethylene at a dry-cleaning establishment (Von Lachnit and Brichta, 1958). Two tests were carried out, bromosulphophthalein clearance and colloid stability. Abnormal results were obtained in eight individuals in one test only, and in three subjects in both tests. However, it appeared that these effects could be related to excessive alcohol consumption rather than exposure to trichloroethylene. No significance can be attributed to these results.

Brief details only have been reported of a later study by the same group involved in measuring serum aminotransferase levels in persons chronically exposed to trichloroethylene (Von Lachnit and Pietschmann, 1960). Of the 31 workers examined, values in the pathological range were noted in only three subjects. Ingestion of alcohol (40 ml as brandy) was shown to result in a

marked increase in serum aspartate aminotransferase levels in those workers exposed to trichloroethylene; this dose had no effect in individuals not exposed to trichloroethylene. Unfortunately, insufficient details were given to allow a meaningful assessment of the significance of these results.

In another report, 14 cases of trichloroethylene exposed workers with evidence of liver disease were described (Schuttmann et al., 1970). In no case were the results complicated by alcohol or drug abuse, malnutrition or any disorders of metabolism such as diabetes. Only brief details were given regarding exposure conditions. It was stated that in ten of the cases atmospheric levels of trichloroethylene at work had exceeded the East German MAC value (50 $mg.m^{-3}$ - 9 ppm), but no further details were available. It was known that the patients had experienced symptoms that could be attributed to trichloroethylene toxicity (vertigo, headaches, tiredness, nausea, vomiting and loss of appetite). Evidence of liver damage included three cases of enlarged liver, raised serum enzyme levels (aspartate and alanine aminotransferases, and aldolase), raised serum gamma-globulin levels, impairment of function (as determined in a bromosulphophthalein clearance test) and fatty deposits in biopsies samples. However, it is not possible on the basis of the available information to determine if the liver changes were in any way related to trichloroethylene exposure.

Some evidence of slight impairment of liver function was noted in 70 workers (including 43 women) occupationally exposed to trichloroethylene (Graovac-Leposavic et al., 1964). The subjects worked in factories and used trichloroethylene either for degreasing purposes or for cleaning textiles. Limited data on atmospheric trichloroethylene levels in the factories using degreasing tanks indicated that these were very variable depending on the sampling position; values in the range 93 to 743 ppm were obtained. The corresponding values where trichloroethylene was used for cleaning textiles were 279-1,078 ppm. Each individual was subjected to medical and clinical examination, aimed primarily at investigating potential hepatotoxicity. The following methods of assessing liver function were carried out: thymol turbidity test, flocculation test and cephalin-cholesterol test. No control group was included.

Physical examination revealed slight liver enlargement in 14 individuals (20%), and some abnormalities in the liver function tests were noted in 23 persons (33%). These appeared to be related to length of exposure, being noted in only 2 (11%) of the 19 subjects with under one year of exposure, as compared to 9/19 (47%) in those with over ten years of exposure. Most changes were in the thymol turbidity test, but were regarded as mild. Serum bilirubin levels and total serum protein levels were all within the normal limits. However, electrophoretic examination of serum proteins showed some decrease in serum albumin levels in 28 (40%) of the workers. This also appeared to be dependent on the duration of exposure.

These results suggest that prolonged exposure to levels of trichloroethylene of the order of several hundred ppm could produce some slight liver impairment. However, no control group was used, and the mild hepatotoxic effects noted may have been due to factors other than exposure to trichloroethylene. Consequently, no firm conclusions can be drawn from this study about the effects of occupational exposure.

No evidence of any liver damage was seen in an investigation of a small group (12) of workers involved in degreasing operations at a factory in Paris (Tolot et al., 1964). All the individuals had complained repeatedly of subjective CNS-related symptoms. Atmospheric trichloroethylene levels in the range 167 to 558 ppm were reported, but no information on the method or frequency of sampling was provided. Mean urinary trichloroacetic acid concentrations, measured in 17 workers over a 5 year period ranged from 219 mg/l to 505 mg/l. Tests for liver damage were limited to a flocculation test, measurement of the electrophoretic pattern of serum proteins and

measurement of prothrombin levels. No abnormalities were noted in any individual. In addition physical examination revealed no signs of liver damage. Thus, in this limited study, there was no evidence for any hepatotoxicity in a small group of men, probably with relatively high trichloroethylene exposure and reporting subjective CNS symptoms.

In a study to evaluate the effects of exposure to trichloroethylene, environmental and medical surveys were conducted for both at a plant using trichloroethylene and in the surrounding community (Landrigan and Kominsky, 1987). Personal breathing zone air samples for degreasers in the plant were assessed on two occasions (in February and May of the same year) and the ensuing data were used to select exposed workers for the study. Age-, sex- and race-group-matched controls were selected from office staff. Pre- and post-shift spot urine samples were collected to analyse for trichloroacetic acid and trichloroethanol, to assess absorption of trichloroethylene, at the same two times as the personal air monitoring occurred and liver and kidney function were assessed both times by clinical chemical examination of mid- and post-shift blood samples. At both times, questionnaires were administered to both exposed and comparison subjects to evaluate neurological effects including drowsiness, dizziness, weakness, tremor, loss of co-ordination and mental confusion.

The results of the first evaluation (February) showed that among degreasers, 8-hour TWA values for trichloroethylene ranged from 22-66 ppm (mean 38 ppm), with short-term peaks (5-15 min) ranging from 77 to 370 ppm. Eight-hour TWA values for other workers ranged from 0.1 to 23 ppm. In the pre-shift urine samples at this time, the mean concentration of total trichloroethylene metabolites was 297.5 mg/l, and the mean post-shift value was 479.9 mg/l. Trichloroethanol concentrations rose from 97.9 to 155.2 mg/l during the day, but no increase was apparent over the shift for urinary trichloroacetic acid (pre-shift = 29.5 mg/l, post-shift, 29.4 mg/l).

Medical evaluations at this time were conducted on 9 of 12 exposed workers and 9 control subjects. Their mean ages were 42.7 and 46.4 years, respectively and the mean duration of employment were 4.4 and 9.4 years, respectively. The medical examiners were aware of the workers' exposure. Symptoms (fatigue (7), light headedness (4), sleepiness (4), eye irritation (4), shortness of breath (4), dyspnoea on exertion (3), nausea (3), skin irritation (2), cough (1) and headache (1) were reported by seven of the exposed but none of the control workers. “Cold degreasers” reported fewer symptoms than did liquid-vapour degreasers.

By the time of the second investigation, three months after the first, exposure levels had been markedly reduced (8-hour TWA values for the “exposed” group ranged from 6.9 to 26 ppm (mean 16 ppm), and short-term peak values had decreased to give a mean value of 74 ppm. The concentration of trichloroethylene metabolites in urine was reported to be correspondingly reduced, but no data were given. In this second survey, the numbers of exposed workers reporting symptoms (6 out of 9), and the number of symptoms reported (fatigue (4), light headedness (1), sleepiness (6), eye irritation (3), shortness of breath (1), dyspnoea on exertion (0), nausea (2), skin irritation (1), cough (2) and headache (1)), were marginally reduced. The results of this study indicate the presence of subjective CNS and irritation-related symptoms in trichloroethylene exposed workers, but because only small numbers of workers were investigated, no definitive conclusions can be drawn from this study.

A case is described of a 51-year-old woman with polyneuropathy assumed to be due to exposure to trichloroethylene in Japan (Takeuchi et al., 1986). The woman had been exposed to high concentrations of trichloroethylene for about 12 years in her employment dipping baskets of metal parts into a warm (80°C) bath of trichloroethylene (>99% pure) for 5 to 6 hours per day, 6 days per week. She also cleaned the trichloroethylene bath twice a week in an operation which

took 15 minutes. Measured atmospheric concentrations of trichloroethylene were found to be 579 to 792 ppm in the normal breathing zone, but when, as was her habit, she was bending over the bath, and during cleaning operations exposures were as high as 2,099 ppm in the breathing zone. No respirator or gloves were provided.

Soon after starting this employment she experienced abnormal fatigue and sleepiness and suffered frequent headaches. She often felt drunk and dizzy during the cleaning of the trichloroethylene bath. After 2 years, she began to experience coolness in the feet and hands. After seven years, she noticed distal dominant paresthesia in her feet and hands and around the mouth, and a sensation of swelling in the soles of her feet. Her feet became very painful. She became hypersensitive to daylight and sometimes suffered from double vision. She became dyspnoeic at night and found her grasping ability was reduced.

Medical examinations revealed that the muscle strength and tendon reflexes of her extremities were severely weakened and her co-ordination was slightly clumsy and slow. A slight delay in motor nerve conduction velocity was apparent. Clearly, this woman, who had been exposed to very high concentrations of trichloroethylene, had chronic central and peripheral nerve impairment.

Two cases of occupational exposure to trichloroethylene associated with alcohol intolerance as the sole symptom have been described (Sbertoli and Brambilla, 1962). The first individual had been degreasing metal parts over an unventilated tank of trichloroethylene during the fortnight before admission. He reported headaches, asthenia, nausea and redness of the face and neck after ingestion of even small quantities of wine with a meal. He had always been a “light” drinker. On admission, he had 424 mg/l of trichloroacetic acid in his urine. The second subject was also degreasing metal parts over unventilated tanks for a few days three years before and then for the two days prior to admission. On both occasions he had experienced feeling hot straight after meals during which he regularly consumed 0.25 litres of wine. He was red in the face for about an hour. Trichloroacetic acid was detected in his urine at a concentration of 374 mg/l. No effects were observed in either case when a glass of wine was consumed at the clinic.

It is postulated that there is competitive inhibition of acetaldehyde dehydrogenase leading to an accumulation of acetaldehyde in the blood following alcohol consumption by certain people exposed to trichloroethylene Similar symptoms have been observed following administration of acetaldehyde itself. It is suggested that acetaldehyde produces acetyl radicals, thereby increasing the production of acetylcholine leading to vagal hypertony. This, in turn, may cause vasodilation, lower arterial pressure and compensatory tachycardia.

Alcohol intolerance was also reported by workers at a plant where trichloroethylene was used for degreasing metal parts (Pardys and Brotman, 1974). One worker studied showed facial flushing, a sensation of increased pressure in the head, lachrymation, tachypnoea and blurred vision within 12 minutes of drinking 85 ml of bourbon whiskey. The unpleasant effects following alcohol consumption occurred in these workers at weekends, but cleared after a week free from exposure to trichloroethylene. Removal of trichloroethylene from the workplace resulted in complete cessation of these responses in all the affected workers.

Summary of studies of general health and symptoms

A comprehensive, but briefly reported, study in volunteers, involving pulmonary function tests, EEG, haematology and blood clinical chemistry investigations and behavioural testing found no evidence of toxicity. The volunteers were exposed to atmospheric concentrations of up to

200 ppm for up to 7.5 hours a day. However, a reaction to alcohol, characterised by a marked but transient vasodilation of the skin vessels, was found in subjects exposed to 200 ppm.

Although there are numerous reports of health surveys being carried out on workers occupationally exposed to trichloroethylene, their value is severely limited by the lack of any detailed information on the atmospheric trichloroethylene levels, exposure to other chemicals including alcohol and on potential confounding factors. Furthermore, in many cases, no control group was used. Consequently it is difficult to assess either the qualitative or quantitative relationship any observed health effects or symptoms may have with trichloroethylene exposure.

Most studies report the presence of subjective symptoms of CNS disturbance in exposed workers. Consistently reported symptoms include fatigue, vertigo, dizziness, headaches, memory loss and impaired ability to concentrate. Also, there are a number of reports of skin and eye irritation. Although the consistency of the reports lends support to the view that these symptoms were related to trichloroethylene exposure, the available data do not allow conclusive judgements to be made regarding causal or dose-response relationships.

Intolerance to alcohol, presenting as a transient redness affecting mainly the face and neck (generally known as “trichloroethylene or degreasers' flush”) has also been frequently observed. Identical effects have been seen in single and repeated dose volunteer studies, confirming that this effect is caused by trichloroethylene.

Some studies included an investigation for possible liver effects. Evidence of the presence of liver damage in trichloroethylene-exposed workers was seen in certain studies, seen as by hepatomegaly or changes in blood clinical chemistry parameters. However, such finding were not seen other studies, including one involving workers who had experienced relatively high exposure levels. Overall, there were no consistent, convincing evidence of trichloroethylene-related liver damage.

Specific neurological investigations

- Studies in volunteers

- Electroencephalograph studies:
The effects of trichloroethylene exposure on the EEG pattern were investigated in volunteers (Konietzko et al., 1975). A total of 20 healthy male subjects (students and scientific assistants), average age 27 years, were exposed to an atmospheric concentration of 95 ppm trichloroethylene. It is not stated whether the volunteers had any previous exposure to trichloroethylene. The subjects spent the mornings, in groups of four, in the exposure chamber. The EEG was recorded for one minute every hour whilst the subject had his eyes closed, and all external stimuli were excluded. The first set of readings was taken under control conditions; the second readings were taken exactly one week later, during which time the individual was exposed to mean levels of trichloroethylene of 95.3 $\pm$ 8.2 ppm (monitored continuously), for a period deduced to be four hours. The emphasis in the EEG tracings was on alpha wave activity; the duration, amplitude and frequency were noted.

The EEG pattern found in the volunteers under control conditions revealed no marked abnormalities. No significant changes were noted when the same individuals were exposed to trichloroethylene. A slight increase in the duration of alpha activity was noted during the first two hours of exposure only: no effects were noted on the amplitude or frequency of these alpha waves and the changes were minor and considered to be of no biological relevance. Thus, in this study, no significant EEG changes were noted in volunteers exposed to about 95 ppm of trichloroethylene for four hours.

EEG investigations were also conducted as part of a previously described study (Stewart et al., 1974a). No significant changes were found in volunteers exposed to atmospheric concentrations of up to 200 ppm for 7.5 hours a day. It should be noted that no data were presented in the report, so it was not possible to validate the authors' conclusion.

- Studies using behavioural tests:
The effects of exposure to trichloroethylene at 100 ppm on performance of a range of tests designed to measure a number of psychological/psychomotor parameters has been investigated (Nakaaki et al., 1973). Only four subjects were used in this study. Exposures to 100 ppm of trichloroethylene were for six hours a day (with a one-hour break after the first three hours) on four successive days. Each subject performed a range of tests during and after every exposure period. These included a simple reaction time test, hand steadiness test, a time estimation test and the critical flicker fusion test. Control values were obtained when the same individuals were subjected to identical conditions, over a separate four-day period, but with no exposure to trichloroethylene. Exposure to 100 ppm of trichloroethylene for six hours daily for four days produced no significant impairment in performance in any of the tests.

A similar trichloroethylene concentration has been used in another study, again using small numbers of volunteers (Triebig et al., 1977a). The exposed group consisted of three male and four female students aged 19 to 28 years. They were exposed to 100 ppm of trichloroethylene for six hours a day (8 am to 2 pm) on five successive days. During each exposure period a range of behavioural tests were performed. The results obtained were compared to those found in a similar group exposed to a “placebo” atmosphere produced by a mixture of hair lotion and disinfectant.

Tests designed to measure a wide range of psychomotor/psychological functions, were performed twice during each exposure period (once near the beginning and once near the end). These tests included the “d-2 test” designed to measure attention concentration and perseverance, and the “short syndrome” series of nine tests demonstrate perceptive and cognitive function. Full details of the actual tests were not given. In addition, an assessment of mood/anxiety state was made. No significant differences were reported in the performance of any of the behavioural tests between the exposed and the control groups. However only brief details of the overall results obtained in each series of tests were given; the results of individual tests were not available. A full assessment of this work is thus not possible.

The only significant difference noted between the exposed and control subjects was in anxiety state. Anxiety levels fell to a greater extent in the control group during the course of the investigation. However, the two groups were not strictly comparable in this aspect, since the individuals in the group exposed to trichloroethylene knew each other prior to the start of the study, whereas those in the control group did not. Therefore, social factors may have accounted for this difference.

Thus, in this study in a small group of volunteers, no impairment in performance of a range of behavioural tests was noted in subjects exposed to 100 ppm of trichloroethylene for six hours a day for five days. However, insufficient details were given for a full assessment of the work.

Behavioural tests were also conducted as part of a previously described study (Stewart et al., 1974a). No significant changes in performance were found in volunteers exposed to atmospheric concentrations of up to 200 ppm for 7.5 hours a day.

- Studies in occupationally exposed groups

- Electroencephalograph studies:
EEG recordings have been made on workers whilst operating a trichloroethylene degreasing tank (Konietzko et al., 1973). A total of six workers were investigated at two separate factories. Trichloroethylene levels were monitored by sampling near the breathing zone during the shift. These were usually below 100 ppm, with an average exposure of about 50 ppm, except in one case where the average was near 100 ppm, with a maximum of about 115 ppm. The EEG recordings were made using a telemetric procedure, with readings being taken every hour during the shift, when the subject stood or sat for a few minutes with closed eyes. The results obtained were compared with those found in three of the individuals when subjected to work of similar physical activity, but not involving exposure to trichloroethylene.

In four of the six workers exposed to trichloroethylene, an increased appearance of alpha waves was noted. This alpha phase activity was usually of about 1 to 2 seconds duration, the amplitude increasing during exposure, but the frequency remaining unchanged. In no case was any theta or delta activity noted. Alpha activity was weaker or absent during exposures under control conditions. These results, in a very small group of workers, suggest that very minor changes in the EEG pattern occur in workers exposed to about 100 ppm of trichloroethylene. The health significance of the slight change is, however, very doubtful.

- Investigations of motor nerve conduction velocity:
Recently, nerve function has been assessed in a group of printing workers with long-tern exposure to trichloroethylene (Ruijten et al., 1991). The study population consisted of 31 male printing workers with at least 6 years of exposure to trichloroethylene, and 28 non-exposed workers from the same printing works. Workers with other risk factors for neuropathy and those consuming more than 50 glasses of alcohol a week were excluded. Controls were matched with cases for physical job activity, education, nationality and age. All exposed subjects had worked with an ink containing trichloroethylene. Three years before this investigation, the work was relocated and a water-based ink was introduced. At the time of the study, trichloroethylene-based ink was therefore used to a much lesser extent than previously. Current mean trichloroethylene exposure levels were estimated to be about 17 ppm. Three years previously, it was estimated to have been about 35 ppm for eight years following the introduction of local exhaust ventilation. Prior to that, trichloroethylene exposures were estimated to have been 70 ppm. It is not stated if these values are 8-hour TWAs. Individual cumulative exposures were calculated, based on work history. Neurological function tests (of autonomic, trigeminal and peripheral nerves) were carried out by examiners blind to the exposure status of the subjects. The workers also completed a questionnaire on drinking habits, past diseases and exposure to other factors which might cause neuropathy and neurological symptoms.

The results showed a slight reduction in sural nerve conduction velocity (-1.1 m/second) and a 0.4 millisecond (ms) prolongation in the sural nerve refractory period in the exposed workers compared with the controls. Masseter reflex (a measure of trigeminal nerve function) was increased by 0.4 ms in exposed subjects but there was no prolongation of blink reflex (another indicator of trigeminal nerve function). There was no impairment of the motor functions of peripheral nerves or of autonomic nerve function. The questionnaire responses revealed no symptoms of neuropathy. Although slight changes were detected in trichloroethylene exposed workers, the physiological significance of these are unclear and, from the data presented, they cannot be clearly related to specific exposures (levels or duration) to trichloroethylene.

Another investigation involved a very small group of workers (two women, five men) in a printing works (Triebig et al., 1978). Their age range was 19 to 32 years and their duration of

exposure varied between six months and nine years. The average environmental trichloroethylene levels to which the workers were exposed at the time of the study were 20 to 40 ppm. The results obtained were compared with those found in a small control group (four women, nine men) stated to be of comparable age to the exposed workers. No further details were given.

Clinical examination and enquiry revealed no signs of neurological illness in any of the workers. The maximum and the minimum motor nerve conduction velocity were measured, using both the ulnar and the radial nerves, in each worker. The values found in the exposed workers for the maximum nerve conduction velocity in the ulnar were in the range 52.8 to 63.9 m/second and those for the minimum conduction velocity were 38.5 to 57.9 m/second. These were not significantly different from the values found in the control group, nor from normal values found by other workers. Similarly, no difference was noted between the exposed and the control group in either maximum or minimum conduction velocity measurements in the radial nerve. Thus, in this study, using very small numbers of workers exposed to mean trichloroethylene levels of up to 40 ppm, nerve conduction velocity measurements gave no evidence of any peripheral neurotoxicity.

In further work from this group, thirty-one employees (26 men and 5 women, aged between 16 and 56 years) from three firms were examined (Triebig et al., 1982). They had been exposed to trichloroethylene for periods of from 1 month to 35 years (mean = 7 years). Twenty-four sex- and age-matched control subjects with no history of, or risk factors for neuropathy, were used. An unknown number of random air samples were taken in which the concentrations of trichloroethylene ranged from 5 to 70 ppm (no further details were given). Peripheral nerve function was assessed on the basis of electromyographic measurements of nerve conduction velocity. No statistically significant differences were observed between those exposed to trichloroethylene and the non-exposed people. None of the subjects reported any symptoms of peripheral neuropathy.

The maximum nerve conduction velocity of the ulnar nerve was investigated in a small group (17) of workers, aged 26 to 50 years, who had reported symptoms of trichloroethylene toxicity whilst working at various different factories in Poland (Kotwica and Szulc-Kuberska, 1973). The subjects were mainly women (14/17), and they had been occupationally exposed to trichloroethylene for between two and 20 years at levels said regularly to exceed the national MAC concentration (not given, but probably 50 mg/m^3; 9 ppm) by two to ten times. The principal symptoms noted were dizziness, headaches, fatigue, irritability, sleep disturbances, and reduced appetite. Paraesthesia in the extremities was noted in three of these workers and facial sensory loss in one.

Values obtained for the motor nerve conduction velocity in the exposed workers were in the range 55-62 m/s. These values were stated to be in the normal range expected, but no control group was used for comparison purposes. Electromyographic measurements were obtained from a small sub-group (five workers); there was no evidence of any abnormal response.

Overall, this study, involving only a small number of workers, provided no evidence of any impairment of nerve conduction velocity in subjects with symptoms of trichloroethylene toxicity; however, the study is of little value because of the absence of a control group.

In summary, the effects of occupational exposure to trichloroethylene on motor nerve conduction velocity have not been extensively investigated. However, the available studies provide no evidence for any peripheral neurotoxicity in persons occupationally exposed to trichloroethylene.

- Studies using behavioural tests:
The effect of repeated exposure to trichloroethylene on the performance of a range of behavioural tests has been investigated occupationally exposed groups trichloroethylene.

A study to investigate solvent-related neurotoxicity has been conducted on a population of 96 out of 99 Danish workers who had used either trichloroethylene or CFC 113 for degreasing (Rasmussen et al., 1993b). Details of the workers, their exposure and how they were split into exposure-matched groups are given above (Rasmussen et al., 1993a). No control group was included. Age, alcohol abuse (defined as above, see Rasmussen et al., 1993a), primary intellectual level, certain disease history and current (compared with long-term) solvent exposure history were determined. The assessment of degree of neurosis or "psycho-organic syndrome" was based on the non-blind taking of a medical history of symptoms of mental impairment, performance in various tests and clinical signs of demential behaviour, including how the subject coped with the test situation. The psychological examinations and assessments were conducted blind. Atmospheric trichloroethylene levels had not been determined; however, current urinary trichloroacetic acid concentrations were reported for the "high" (mean exposure of 11 years) of mean 7.7 mg/ml, with a maximum of 26.1 mg/l.

The psychometric tests used were WAIS vocabulary, simple reaction time, acoustic-motor function, discriminatory attention, sentence repetition, PASAT, digit span, text repetition, Rey's auditory verbal learning, visual gestalts, stone pictures (a non-validated visual memory test), the digit symbol test from WAIS, revised Santa Anna Dexterity, motor function ad modum Luria, Mira (psychomotor ability without optical control).

"Psycho-organic syndrome" was defined by the authors as a mild syndrome characterised by cognitive impairment, personality changes and reduced motivation, vigilance and initiative. Diagnosis depends on a characteristic symptom pattern and test results. The degree of syndrome ("demential score") is grouped into no, suspect, mild, mild to moderate, moderate, moderate to severe and severe. "Suspect" applies to subjects with a heavy load of neuropsychological complaints but reduced performance in only a few psychological tests.

The prevalence of "psycho-organic syndrome" was 10% for low exposure, 39% for mid exposure and 63% for high exposure. After adjustment for known potential confounders, logistic regression analysis showed an increased risk of developing "psycho-organic syndrome" only for those in the highest exposure group; the odds ratio (OR) was 11.2 (95 % confidence interval = 1.9 to 66.6). Of the 42 workers diagnosed from test results to have "psycho-organic syndrome", 31 had been predominantly exposed to trichloroethylene and seven had been exclusively exposed to trichloroethylene. The risk of developing "psycho-organic syndrome" increased with age (independent of duration of exposure) and with decreasing primary intellectual level (performance in WAIS vocabulary test). No significant associations were found with the other potential confounders (i.e. arteriosclerotic disease, neurological/psychiatric disease, alcohol abuse, current solvent exposure).

The observation of an increased risk of developing "psycho-organic syndrome" being associated with "high exposure" to trichloroethylene cannot, however, be clearly and specifically attributed to trichloroethylene because only a few of the workers were exposed exclusively to this solvent. In addition, no relationship could be drawn between symptoms/test results and actual exposure levels.

Eight workers exposed to trichloroethylene at a screen offset printing works, were subjected to a range of tests designed to measure psychological/psychomotor performance (Triebig et al., 1977b). The mean atmospheric levels of trichloroethylene in the room air at various locations were about 50 ppm. The exposed group consisted of seven men and one woman, their age range

being 23 to 38 years. Each individual was subjected to a range of behavioural tests shortly after the end of the workshift on Friday afternoon, and prior to work on the following Monday. The tests were repeated six weeks later immediately prior to and then after a 15-day vacation period. The tests included the "d-2 test", the MWT-A test (a short intelligence test) and short tests to measure motor performance, recognition and memory. Mean urinary trichloroacetic acid levels at the time of the first batch of tests (at the end of the Friday workshift) were 140 μg/mg of creatinine; values the following Monday morning were 88 μg/mg of creatinine. The values obtained in the second series of tests were 153 μg/mg of creatinine and 25 μg/mg of creatinine before and after the vacation, respectively.

No significant differences were seen in the performance of any of these tests, at any time. The results obtained in the d-2 tests were somewhat complicated by a slight improvement being noted during the series of tests, due presumably to a training effect. The results obtained were believed to be comparable to those expected in a normal population, but no control group was included for comparative purposes.

No significant effects on performance of a range of behavioural tests were noted in this study in workers occupationally exposed to mean atmospheric levels of trichloroethylene of about 50 ppm. However, the value of this study was limited by the small number of subjects used and absence of control group.

The reaction times of small groups of women workers, involved in the operation of trichloroethylene degreasing baths at two separate Australian factories, have been investigated (Gun et al., 1978). Again, only small total numbers were involved (four workers at each site). Mean breathing zone concentrations of trichloroethylene near the degreasing tanks were about 245 ppm (range 148 to 418 ppm) at one factory and 27 ppm (range 3 to 87 ppm) at the second factory. The study was apparently prompted by complaints of symptoms of drunkenness in some women operating the degreasing bath; it is not clear whether these complaints were solely from the first factory, or whether they had occurred in workers from both sites.

Each worker performed a complex reaction time (eight-choice) test during four separate sessions. These were prior to and at the end of the morning shift (four hours), and at the beginning and end of the afternoon shift. The results obtained were compared with those obtained from control groups, each consisting of four women workers from other areas of the factories, where trichloroethylene was not used. No further details of the comparability of the exposed and control groups were given.

The initial mean reaction times were comparable in the exposed and control groups, being 102 to $103 \cdot 10^{-2}$ seconds at the first factory and 95 to $97 \cdot 10^{-2}$ seconds at the second factory. Reaction time decreased somewhat during the day in both the control groups, showing some improvement in performance, presumably due to a training effect. However a marked increase in reaction time was noted during the day in the exposed workers at the first factory, where the environmental trichloroethylene levels were highest. In these workers mean reaction times at the end of the day were $124 \cdot 10^{-2}$ seconds, compared with $96 \cdot 10^{-2}$ seconds in the control group at the comparable time. No increase in mean reaction times was noted during the day in the workers at the second factory and only a small difference was noted between the exposed and control groups at the end of the day ($98 \cdot 10^{-2}$ seconds, compared with $89 \cdot 10^{-2}$ seconds).

These results suggest that exposure to mean trichloroethylene levels of about 250 ppm resulted in some reduction in performance in reaction time tests. Symptoms of marked CNS disturbances (drunkenness) had been noted in these workers. No marked impairment was noted in workers exposed to up to about 90 ppm trichloroethylene. However, no details were given as to the

comparability of the control and exposed groups regarding age, physical activity during the work shift, or any other factors. The significance of the results reported cannot therefore be fully assessed.

Summary of specific neurological investigations

The potential neurotoxicity of trichloroethylene has investigated using specific tests in only a limited number of studies. These have been conducted in volunteers, receiving short-term repeated exposure and occupational cohorts with long-term exposure.

In volunteers, no evidence of biologically significant effects on EEG patterns or on performance in behavioural tests were seen studies involving exposures of up to 200 ppm, for up to 7.5 hours. In trichloroethylene exposed workers, the available studies provided no evidence of significant effects on nerve conduction velocity or EEG patterns. Effects on performance in neuropsychological and reaction-time tests have been reported in two studies, but the data were too limited to draw conclusions about their relationship with trichloroethylene exposure.

Overall, the neurotoxicity of trichloroethylene has not been extensively evaluated in humans.

Effects on kidney

The toxicity of trichloroethylene to the kidney has been investigated in German workers. Two studies were conducted in connection with investigations into the possible association between occupational exposure trichloroethylene and renal cell cancer.

In a briefly reported study, considered by the authors to be a preliminary investigation, the presence of unusual patterns of protein excretion was investigated in 17 cases of renal cell carcinoma in workers who had been occupationally exposed to trichloroethylene (Brüning et al., 1996). The mean duration of exposure was 15.5 years and a mean latency period from start of exposure to cancer diagnosis was about 30 years. Exposure was considered to be higher than currently experienced in workplaces using modern standards of occupational hygiene. For comparison, protein excretion was conducted in 35 renal cell carcinoma patients without occupational exposure to trichloroethylene. Investigations were conducted after the patients had undergone nephrectomy; the time period between nephrectomy and investigation was unstated, but said to be comparable for exposed and non-exposed patients. Protein patterns were investigated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), which, according to the authors, allows the semi-quantitative determination of 20 different urinary proteins. Diagnosis of tubular or glomerular damage were made by a clinical nephrologist who was blind to exposure status on the basis of excreted proteins, using unspecified criteria. Tubular or glomerular damage was reported in all trichloroethylene-exposed patients and 51% of non-exposed patients. The results of this study suggest that there may have been a higher prevalence of kidney damage in the trichloroethylene exposed patients. However, because the diagnostic criteria are not known, and information on other factors, such as age, which may influence protein excretion were not available, no firm conclusions can be drawn from this study.

In a further study, urinary excretion of α-1-microglobulin, glutathione transferase (GST) α (both said to be markers of proximal tubular damage) and GST π (said to be a marker of distal tubular damage) was investigated in 39 trichloroethylene exposed male workers (Brüning et al., 1999). Additionally, serum creatinine, serum urea, urinary creatinine and total urinary protein were measured. The workers were from a factory in which trichloroethylene was used for degreasing from 1956 to 1975; the study was conducted in 1995. Average duration of exposure was 16 years

(range 6-20). Selection criteria were not reported, so it is not possible to judge if this procedure introduced bias. None were diagnosed with cancer. Exposures to trichloroethylene were ranked semi-quantitatively by applying a system that integrates total exposure time and frequency and severity of acute symptoms. The highest exposed workers were thought to have experienced intermittent exposures in excess of 500 ppm, based on reported symptoms. A control group comprised 46 male office workers from the same factory, who had no trichloroethylene exposure. The age ranges and smoking habits of the exposed and control groups were similar. Urinary GST proteins were analysed using SDS-PAGE. Among the trichloroethylene exposed workers, urinary excretion of α–1-microglobulin and GST α were significantly increased (2-3-fold) in comparison with the control group, although there was no relationship with exposure level. Urinary GST π, serum creatinine, serum urea, urinary creatinine and total urinary protein were similar for exposed and control subjects. The health significance of the observed changes is uncertain, so it is not possible to draw any firm conclusions about the adverse health effects of trichloroethylene on the kidney from this study.

Kidney toxicity has also been investigated, albeit in a limited manner, in the previously summarised study of Rasmussen et al. (1993). This study did not provide any evidence of kidney toxicity.

Cardiovascular effects

- Case history

The cardiac arrest suffered by a 44-year-old male has been attributed to repeated occupational exposure to trichloroethylene (Wernisch et al., 1991). The patient collapsed while mowing a lawn. After admission to hospital, he suffered frequent polytopic ventricular extrasystoles and tachycardia. The biological cause of the cardiac arrest was diagnosed as ischaemia of the "front wall" from the ECG, but serum transaminase levels indicated he should not have suffered a myocardial infarct. It was later established that, for many weeks prior to the incident, he had suffered episodes of amnesia and confusion, a burning sensation in the pharynx and eczema of the hands, underarms and head. He had recently been working 10 hours per day in a poorly ventilated workshop using rapid setting adhesives in shoemaking. He did not smoke or drink alcohol or suffer from known heart disease. Serum enzyme analysis revealed some signs of liver injury, but heart- and kidney-specific enzyme levels were normal. Fourteen days after his last day at work, his urinary excretion rate for trichloroacetic acid was 2 mg/24 hours. He had several neurological symptoms which resolved only very slowly. However, whether there is a cause-and-effect relationship in this case between exposure to trichloroethylene and cardiac arrest cannot be established.

- Studies in occupationally exposed groups

Electrocardiographic examination was included as part of a health survey of a group of 30 workers at an Egyptian printing factory; most were below 40 years of age and had been employed for more than three years (El Ghawabi et al., 1973). Mean atmospheric levels of trichloroethylene were said to be in the range 41 to 163 ppm at different parts of the plant at the time of the study. In this study an increased incidence of subjective CNS symptoms, together with signs of irritancy to the skin and eyes, was seen in exposed workers. No abnormalities were seen in the ECG recordings.

Electrocardiograms have also been recorded from 77 workers occupationally exposed to trichloroethylene in various industries in Sweden (Anderson, 1957). Symptoms of marked CNS disturbances were noted in many of these workers. Pathological ECG tracings were noted in approximately one-third of those studied; these were mostly indicative of disturbances in cardiac autonomic mechanism. It was suggested that abnormalities such as ectopic auricular rhythm, shifting pacemaker and perisinus rhythm were sequelae of exposure to trichloroethylene, whilst the disturbances in the conduction system were not related to exposure. However, insufficient information is available to determine whether any of the effects were related to exposure to trichloroethylene.

Haemodynamic measurements, and electrocardiograms were recorded during examination of 44 workers (mainly women below 30 years of age) exposed to trichloroethylene at a Romanian factory (Lilis et al., 1969). Although it was reported that routine atmospheric monitoring only occasionally gave readings above 37 ppm, pre-narcotic symptoms had commonly been experienced by the workers, suggesting the exposures were actually considerably higher.

Electrocardiography revealed a few abnormalities, but these were believed by the authors to be related to pre-existing heart conditions rather than exposure to chemicals. Comparison of the systemic haemodynamic measurements (Broemser Ranke physical method) obtained in the exposed workers with the results obtained using a small control group of ten subjects revealed a number of differences. The control group was stated to be of comparable age and sex to the exposed workers but no further details were given. A significant increase was noted in stroke volume, cardiac output, cardiac index and heart work in the exposed workers. The values

obtained were 84.4 ± 18.9 ml, 6,615 ± 1,640 ml/l, 4,036 ± 1,100 ml/l/ml and $10.86 \pm 2.84 \cdot 10^8$ ergs respectively in the exposed workers, compared with 68.4 ± 5.4 ml, 4,830 ± 710 ml/l, 3,077 ± 479 ml/l/ml and $7.84 \pm 0.91 \cdot 10^8$ ergs in the controls. These increases were considered to reflect an adrenaline-type increased sympathetic tone, which, it was suggested, could play a part in a possible sensitising effect of trichloroethylene on the action of endogenous catecholamines on the myocardium. However, in view of the very limited details given of the control group, no firm conclusions can be drawn from this investigation.

Effects on the cardiovascular system have been specifically investigated in a small group (20) of women workers at a factory in Sofia (Dimitrova et al., 1974). The age range of the subjects was 34 to 47 years and they had been employed for between seven months and four years. The results were compared with those obtained in a control group of 58 healthy individuals of approximately the same age and stated to have "similar work histories but without exposure to toxic chemicals". No further details were given regarding the comparability of individuals in the control group to the exposed workers. No information was given regarding the atmospheric trichloroethylene levels at the factory, apart from the statement that they were above the national MAC value. Mean urinary trichloroacetic acid levels at the time of the study were 40.71 ± 6.61 mg/l in the exposed group and 1.58 ± 0.68 mg/l in the controls, but no details were given as to the time post-shift that these samples were taken. Urinary trichloroethylene levels were also determined in 11 of the exposed workers. The mean value obtained was 45.70 mg/l. However, appreciable amounts were also present in the control workers, a mean value of 28.45 mg/l being obtained in this group. It is thus likely that the control workers had been exposed to significant amounts of trichloroethylene.

Cardiac effects measured in each subject included the simultaneous recording of ECG, carotid sphygmogram and phonocardiogram. In addition, phase analysis of the left ventricular systole was carried out using a polycardiographic method. Significant differences were noted between the exposed and the control group in a number of polycardiographic indices. The cardiac cycle was shortened, the isometric period and the tension phase was prolonged, and the intrasystolic index was decreased. These results suggest some impairment of cardiac function, due to a prolongation of the ineffective phase of systole, in the trichloroethylene workers. However, in view of the lack of details regarding the comparability of the exposed and control groups, the unusual methodology, and the possibility that the controls were also exposed to trichloroethylene, the significance of the cardiac changes noted cannot be assessed.

A cross sectional study to investigate cardiac effects in 75 workers involved in degreasing operations using trichloroethylene at a West German factory has been reported (Konietzko et al., 1975). All the workers were subjected to medical examination, during which an ECG recording was made. The average age of the workers was 43 years (range 20 to 64 years), and the duration of employment was from two months to 20 years. Limited data available on atmospheric trichloroethylene levels suggested that these were about 100 ppm in the vicinity of the degreasing tank. Electrocardiographic recordings were made in a number of the workers at yearly intervals after the initial reading.

Abnormal ECGs were noted in three of the workers. In one case (right-heart block) the history suggested that this had existed for some years prior to working at the factory, and could not therefore be associated with exposure to trichloroethylene. In another case, ventricular extrasystoles were noted in one healthy young worker (aged 29) who had been employed for six months. He was then transferred to another area and further ECGs were not recorded. The third case was a 50-year-old man who had been employed for 20 years. His ECG was normal during the first examination, but two years later signs of first degree AV heart block were present. Clinical and radiological examination revealed no abnormalities in this individual.

Thus, of the three cases of abnormal ECGs noted in the exposed workers, one was known to be unrelated to occupational exposure to trichloroethylene and is not possible, in view of the very limited nature of the study, to assess the significance of exposure to trichloroethylene in the other two instances.

In a second study by the same investigators, ECG recordings of six workers using a degreasing tank were continually monitored, by telemetry, during one workshift (Konietzko et al., 1975). Average atmospheric levels of trichloroethylene at the time were 87 ppm. The results obtained were compared to those obtained in the same individuals during the workshift the next day when they were involved in jobs not entailing exposure to trichloroethylene. Ventricular extra-systoles were repeatedly noted in an apparently healthy 34-year-old man during and after the workshift involving exposure to trichloroethylene. No abnormalities were, however, observed during or after the second work-shift, which did not entail exposure to trichloroethylene. Examination (consisting of medical history plus clinical and radiological examinations) revealed no evidence of ill health in this individual. A further group of six workers was monitored in a similar fashion over a 40-week period whilst exposed to trichloroethylene using the degreasing bath. However, in no case were any ECG abnormalities noted. Overall, this study has shown the presence of trichloroethylene-associated ventricular arrhythmias in some individuals exposed to an atmospheric concentration of around 100 ppm of trichloroethylene. However, in view of the small numbers of individuals investigated, no definite conclusions can be drawn.

Summary of cardiovascular effects

A number of studies have been carried out to investigate the effect of occupational exposure to trichloroethylene on the cardiovascular system, particularly cardiac rhythm. The value of these has however usually been limited both by the lack of data on environmental levels present, and the failure to use an adequately matched control group. Some very limited evidence is available to suggest that exposure to about 100 ppm of trichloroethylene may produce ventricular arrhythmias in a small proportion of the persons exposed. However, the number of subjects affected was very low, and further work is needed to investigate this aspect before any definitive conclusions can be drawn.

Effects on hearing and the vestibular system

There are two studies which investigated ototoxicity and effects on the vestibular system during health surveys of workers exposed to trichloroethylene.

Twenty-two workers from a tannery with a total workforce of 30 men, were investigated for auditory and vestibular defects (De Rosa et al., 1971). A control group was not included. No data were available on the atmospheric trichloroethylene levels to which these workers were exposed, but there was a high incidence in the workforce of symptoms of CNS disturbances and also signs of irritant effects on the mucous membranes. Some impairment in auditory function (hypoacusis at 4 kHz) was noted in six of the workers, but this was likely to have been due to noise; all worked in the drum department which was described as “very noisy”. Vestibular examination revealed abnormalities, described as symmetrical bilateral vestibular hyper-reflexia, in 11 individuals (50%). These were apparently markedly reduced in a few instances where the men were re-examined two months after exposure ceased. Although this study demonstrated the presence of auditory and vestibular impairment in trichloroethylene exposed workers, the study was too limited to allow any conclusions to be drawn about the whether or not these changes were caused by trichloroethylene.

In a separate survey, the results of studies to investigate effects on hearing (audiometric test) and balance (rotary and caloric tests) were reported in 40 workers exposed to trichloroethylene at various factories in Poland (Szulc-Kuberska, 1972; Szulc-Kuberska et al., 1976). The subjects appeared to have been selected on the basis that each had experienced CNS symptoms may have been attributable to trichloroethylene toxicity. The age range of these workers was 25 to 50 years. The only information available on exposure was a statement that all had urinary trichloroacetic acid concentrations of at least 40 mg/l, with some as high as 200 mg/l.

Audiometric tests revealed hearing defects in 26 of these workers (65%); these consisted of perceptive impairment at frequencies in range 2-3 kHz. The frequency of this impairment appeared to be related to the length of exposure, with 13/22 cases (59%) in those employed for 4 to 10 years and 10/11 cases (91%) in those employed for more than ten years. Age, is likely to be a confounding factor. Disorders of balance (revealed as diminished excitability of the vestibular organ) were noted in 19 subjects (48%). Hearing disorders were present in all of these individuals, and most had been employed for over 14 years. However, it is not possible to determine if the hearing and vestibular effects observed were caused by trichloroethylene exposure.

Overall, no conclusions can be drawn from these two studies.

Other effects

A case of diffuse fasciitis with eosinophilia associated with prolonged occupational exposure to trichloroethylene has been described (Waller et al., 1994). However, in this single case, the patient had been exposed to a number of other organic solvents and thus no causal association can be concluded.

There are claims from Japan that the development of primary pneumatosis cystoides intestinalis (PCI), a rare condition characterised by the development of gas-filled cysts in the intestine wall, may be associated with exposure to trichloroethylene (Yamaguchi et al., 1984; Sato et al., 1987). These investigators found that a high percentage (71%) of 21 patients with primary PCI were factory workers who were routinely exposed to trichloroethylene. More studies are need before any conclusions can be drawn about the possible relationship between this disease and trichloroethylene exposure.

Brief details are available of a 37-year-old male who suffered fatal hepatic necrosis following 6 weeks during which he had been degreasing metal parts over a bath of heated trichloroethylene (Preist and Horn, 1965). No information was provided on exposure concentrations or frequency. The individual was admitted to hospital with severe bilateral pain in the flanks. He vomited over the next 6 hours, then began vomiting blood and his temperature rose to around 40^{o}C. He became comatose on the 5th day after admission and died during that day. Necropsy revealed evidence of massive liver damage. There was also evidence of kidney toxicity secondary to liver effects (biliary nephrosis). Other findings included extensive acute inflammation of the trachea and major bronchi, with ulceration and haemorrhage and associated minimal bronchopneumonia. Focal acute oesophagitis and myocardial hypertrophy were also seen. Because only a single case is described, no conclusions can be drawn regarding the association of this death with trichloroethylene exposure.

A woman who worked with trichloroethylene developed anicteric hepatitis (Schattner and Malnick, 1990). She had worked for several years doing cold degreasing for 3 hours per day and was in the vicinity (6 m) of a hot trichloroethylene tank for 2 to 6 hours per day. This tank had no fume hood. There was no exposure to other organic solvents or hepatotoxins. No evidence of

hepatitis A or B was found and there was no evidence of other infectious agents. Nor was there any history of alcohol consumption or use of drugs. At the time of admission, she had a 5-day history of anorexia, recurrent painless vomiting and fever (38.5^{o}C). Biochemical analysis revealed elevated serum alkaline phosphatase, transaminases and 5'-nucleotidase. Her erythrocyte sedimentation rate was markedly increased. The patient recovered over the next 6 weeks but then developed bilateral anterior uveitis. She returned to work 6 months after her illness. Within two weeks, she had symptoms of lassitude and elevated serum alkaline phosphatase levels. The atmospheric concentration of trichloroethylene at this time was 550 ppm. Following removal from working with trichloroethylene, no further symptoms were reported. It is possible that this woman suffered an idiosyncratic reaction to trichloroethylene, but because only a single case is described, no firm conclusions about the causality can be drawn.

Oral

In a study to evaluate the effects of exposure to trichloroethylene, environmental and medical surveys were conducted for both workers in, and the community around a plant using trichloroethylene (Landrigan and Kominsky, 1987). The occupational (inhalation) aspects of this study are addressed above. To screen the community at risk from drinking water contaminated by a spill of trichloroethylene from the plant, 13 residents potentially at high risk of exposure on the basis of high concentrations of trichloroethylene in well water (up to 183,000 ppb in water samples taken within 1 km of the factory, 1 month after the spill) were evaluated four months after the spill occurred. Trichloroethylene was still being detected in well water six years later. It is not clear from the report what the trichloroethylene concentrations were in the water actually consumed and used by the residents studied, but concentrations in residential drinking water wells (taken at an unknown time relative to the incident) ranged to 1,000 ppb. The US EPA estimated that residents of communities near the factory were exposed to drinking water containing more than the EPA proposed limit of 5 ppb, but is not clear whether this was under normal conditions or at some time after the spill.

A questionnaire was used to investigate exposure to trichloroethylene and also to other chemicals; and any signs and symptoms of CNS effects (specifically, headache), liver dysfunction or neuropathy. First morning urine samples were collected and analysed for trichloroacetic acid and trichloroethanol. The lower limit of detection was reported to be 2 μg/l. Two of the 13 residents had measurable levels of trichloroethylene metabolites in their urine. However, the highest concentration (total trichloro-compounds of 615 μg/l) was found in a degreaser who used trichloroethylene at work. The concentration of total trichloro-compounds in the urine of the other resident was 2.5 μg/l, but this person had not consumed any of the contaminated well water and did not work with trichloroethylene, so the source of the urinary substances was unclear. Neither of these individuals reported any symptoms of toxicity and, although it is not stated in the report, it would appear that no other residents reported any symptoms either. Overall, it is not possible to characterise exposure levels and consequently this study does not provide any useful data for assessing the repeated dose oral toxicity of trichloroethylene.

The residual neurological effects of previous exposure to trichloroethylene in drinking water have been studied in humans by measuring the blink reflex latency (Feldman et al., 1988). Clinical neurological assessments were carried out on a group of 28 people from 8 families who alleged chronic exposure to industrial chemicals as a result of contamination of drinking water wells. The results of the investigations showed a highly significant difference in conduction latency ($p<0.0001$) between exposed subjects and a group of non-exposed control subjects,

suggesting a sub-clinical alteration in the Vth (trigeminal) cranial nerve function. However, this study, like many similar studies, is compromised by mixed exposures to unknown quantities of chemicals. Trichloroethylene was detected in the contaminated wells (up to 256 ppb), but so were other chlorinated substances, and it is also possible that other, unidentified, contaminants were present. In addition, the study reported was conducted six years after levels of trichloroethylene (and other contaminants) in drinking water had been reduced to conform with the EPA's enforceable limit (5 ppb for trichloroethylene).

In another report by the same group, three studies (the one above, including further observations, and two others, all in the USA) on the neurotoxic potential of trichloroethylene in drinking water have been reviewed (Feldman et al., 1994). Although diagnoses of various forms of neurotoxicity were made for a significant proportion of the subjects, overall it is not clear what the results show. The exposed subjects were self-referred individuals involved in litigation. There was overt or suspected mixed exposure and quantification of exposure to trichloroethylene is not possible, and the role that other factors could play in the apparent deficits is difficult to assess. Where controls were used they do not seem to have been matched to cases in terms of age or socioeconomic class. The results are therefore inconclusive.

Skin effects

There are a few specific reports of dermal effects in people occupationally exposed to trichloroethylene vapour and/or liquid but it is not clear, if in fact these are related to trichloroethylene, these are local, contact effects or manifestations of systemic effects.

After 30 days of exposure to trichloroethylene while degreasing metal parts, a 23-year- old male developed widespread erythema over his whole body, maculopapules and petechiae with a mild sensation of irritation (Hong et al., 1985). On admission to the clinic, he had mild irritation over the whole body with erythema and maculopapules, haemorrhagic vesicles, petechiae and oedema on his palms, soles, lips, eyelids and lower legs and liver function test results indicating liver involvement. Biopsy of papules on his right forearm revealed hyperkeratosis and spongiosis in the epidermis and infiltration of mononuclear cells. He was treated with antihistamines, antibiotics and steroids and the lesions resolved by 44 days. After recovery, a patch test was conducted with 5% trichloroethylene in olive oil; it was negative. No details of the exposure type, pattern or level were given. As this is an isolated case, it is not possible to make a judgement on the possibility of a causal association with trichloroethylene.

Five cases of Stevens-Johnson syndrome (erythema multiforme major), an autoimmune disorder which can apparently be triggered by chemical exposure, have been reported in trichloroethylene exposed workers in Singapore, in which a link was suspected between development of the syndrome and exposure to trichloroethylene (Phoon et al., 1984). The first two individuals were 17-year-old twin sisters who started work on the same day. One worked full time, without protection, dipping transistor parts into trichloroethylene to clean them and her sister did this job for around one hour a day. The section was not air conditioned and was warm due to the presence of ovens. No information was given on actual exposure levels.

After around three weeks, the subject with full time exposure developed a fever and an itchy rash on her face, which became generalised. Three days later her sister became similarly affected. The first sister also developed jaundice with hepatomegaly and both had abnormal liver function tests, raised serum bilirubin and raised serum glutamic pyruvic transaminase (SGPT).

The third case was a 24-year-old male maintenance worker who worked in a room in which open bowls of trichloroethylene situated next to workstations were used to clean solder joints.

Trichloroethylene concentrations were 39 and 131 ppm in two static samples taken from different parts of the room, and 96 and 169 ppm in two personal samples. After he had worked in this area for four weeks, air conditioning and exhaust systems had been switched off for repair work. Erythema multiforme-like lesions developed over the exposed parts of the body and mouth after about 5 weeks of working on this job. The patient was mildly jaundiced with slight hepatomegaly and had raised SGPT. A skin biopsy showed spongiosis with damaged basement membrane, perivascular mononuclear infiltrate and marked upper dermal oedema. The patient recovered after treatment with systemic steroids. A subsequent patch test with 5% trichloroethylene in olive oil was negative.

The fourth case was a 39-year-old male whose workstation was in an air conditioned room containing a partially enclosed trichloroethylene degreasing tank. He spent around 4 hours/day at the workstation. Trichloroethylene exposure levels in the room were found to be less than 9 ppm, but all 18 production workers in the factory had urinary trichloroacetic acid levels between 21 to 156 mg/l. After three weeks work, he developed a generalised macropapular rash with jaundice and slight hepatomegaly. Serum bilirubin, SGPT and serum alkaline phosphatase levels were all elevated. He recovered and returned to work after one week. By the next day, he had developed erythema multiforme-like lesions and jaundice. This pattern of recovery, return to work and deterioration recurred. He eventually died from liver failure.

The final case was a 17-year-old girl who developed an erythematous rash two weeks after starting work at the same plant as case three, above. As a consequence of the investigation of case three, the plant had substituted other solvents for trichloroethylene wherever possible and tanks containing trichloroethylene were now covered. The trichloroethylene concentration in the atmosphere was 370 mg/m^3 (69 ppm). This patient did use trichloroethylene for cleaning machine parts and was known to have used trichloroethylene to wash her hands on at least two occasions. One week after presenting with symptoms, her condition deteriorated and she was admitted to hospital with jaundice, erythema multiforme-like lesions over the trunk and limbs and hepatosplenomegaly. She also had oral ulceration. No trichloroacetic acid was detected in her urine one week after stopping work, but liver function tests were still “grossly abnormal” (no details given). She eventually recovered and did not return to that workplace. She was not patch tested.

Thus, five cases of serious dermal effects with liver involvement in workers exposed to trichloroethylene, by inhalation and, in at least two cases (numbers three and five), also via the dermal route have been described. All cases occurred within a short time of starting work involving trichloroethylene exposure. Although there is circumstantial evidence to suggest that Stevens-Johnson syndrome may represent a rare idiosyncratic reaction to trichloroethylene, it is not possible without the identification of other similar cases from elsewhere, to judge for certain if this condition can be triggered by trichloroethylene.

Cases of scleroderma have been reported following occupational exposure to trichloroethylene and trichloroethane in Denmark (Flindt-Hansen and Isager, 1987). A 52-year-old man had disabling sclerodactyli with nail fold capillary changes, scleroderma of the hands and forearms and Raynaud's phenomenon. These effects appeared over an 18-month period. He had been cleaning high voltage cables with a trichloroethane spray and a cloth containing trichloroethylene. Although he worked out of doors, he did not protect his hands.

A second case was a 63-year-old male who had severe sclerodactyli with contractures, scleroderma of the hands and forearms and Raynaud's phenomenon with digital ulceration and scarring. He complained of dyspnoea on exercise and marked forgetfulness and vertigo. His symptoms had developed over the past 10 years. Central and cortical cerebral atrophy was

apparent from a CT scan and a lung function test revealed reduced diffusion capacity. He had been exposed initially to trichloroethylene and then later to trichloroethane, as a degreaser.

The final case was a 25-year-old male with sclerodactyli and scleroderma of hands proximal to the digits and Raynaud's phenomenon with digital ulcerations. A scaling erythema was found on the knuckles, elbows and knees. He had dyspnoea on exercise and proximal muscle weakness. His symptoms had developed over the past three years. Lung function tests showed restrictive impairment of lung function with reduced diffusion capacity. Electromyogram and muscle biopsies revealed changes consistent with myositis. For the past four years he had been degreasing items with a cloth dipped into a bucket containing trichloroethylene. He did not protect his hands while he worked and there was no ventilation in the room.

The authors suggest that trichloroethylene may be an aetiological factor in the development of systemic sclerosis in these cases. The possibility that this condition may represent an idiosyncratic reaction to trichloroethylene cannot be ruled out, but without corroborative reports from elsewhere no conclusions can be drawn about causality.

4.1.2.6.3 Summary of effects of repeated exposure

In animals, no standard 28-day or 90-day repeated dose inhalation or oral studies with comprehensive observations are available, although an extensive range of studies has been conducted using these routes of administration. No dermal data are available. The main toxic effects which have been observed following repeated inhalation exposure of animals to trichloroethylene are effects on the liver, kidney, CNS, pulmonary system and on hearing. Liver and kidney toxicity have also been reported following oral administration.

A clear NOAEL for neurotoxicity is 200 ppm for long-term inhalation exposure to trichloroethylene; at higher dose levels there was evidence of mild neurotoxicity, reflected in increases in the midlatency component of the flash evoked potential recorded from the visual cortex of the brain. In the lung, vacuolation of Clara cells was observed in mice exposed to 450 ppm; a no effect level for this change is not available. There is evidence that short-term inhalation exposures of rats to trichloroethylene at high concentrations may give rise to persistent mid-frequency hearing loss; a NOAEL of about 2,000 ppm of trichloroethylene can be identified for this effect.

Trichloroethylene produces a range of changes in the liver (e.g. increased liver weight, increased P450 activities, increased serum enzyme markers of liver dysfunction, fatty infiltration, centrilobular cell enlargement and, at very high doses, centrilobular necrosis) following repeated inhalation or oral exposure of animals to trichloroethylene. The mouse is more sensitive than the rat and, in oral studies, peroxisome proliferation has been shown to occur in the livers of trichloroethylene-treated mice, but not in those of rats. Inhalation and oral NOAELs of 200 ppm and 500 mg/kg/day, respectively were identified.

Regarding the kidney, tubule meganucleocytosis was reported in male rats, but not female rats or mice of either sex in a following long-term inhalation exposure; a NOAEL of 100 ppm was identified for this route. By the oral route, trichloroethylene elicited kidney toxicity (tubular cytomegaly and dilatation) in rat and mouse cancer bioassays at dose levels of 250 mg/kg/day and above; a NOAEL of 50 mg/kg/day was identified.

Overall, in animals, kidney toxicity appears to be the most sensitive endpoint for both long-term repeated inhalation and oral exposure. For inhalation exposure, the NOAEL is 100 ppm. For oral exposure a NOAEL of 50 mg/kg/day can be identified.

In humans, the health effects of trichloroethylene have been investigated in a limited number of small-scale volunteer studies, where exposure was controlled, and in numerous surveys and reports involving occupationally exposed groups.

In volunteers, no evidence of trichloroethylene toxicity has been observed following short-term repeated exposure to atmospheric concentrations of up to 200 ppm for up to 7.5 hours/day; pulmonary function and behavioural tests, EEG, haematology and blood clinical chemistry investigations were conducted. Although there are numerous reports of health surveys being carried out on workers occupationally exposed to trichloroethylene, their value is severely limited by the lack of any detailed information on the atmospheric trichloroethylene levels, exposure to other chemicals including alcohol and on potential confounding factors. Furthermore, in many studies, no control group was used. Consequently it is difficult to assess either the qualitative or quantitative relationship any observed health effects or symptoms may have with trichloroethylene exposure. Most studies report the presence of subjective symptoms of CNS disturbance in exposed workers. Consistently reported symptoms include fatigue, vertigo, dizziness, headaches, memory loss and impaired ability to concentrate. Also, there are a number of reports of skin and eye irritation. The consistency of the reports lends support to the view that these symptoms were related to trichloroethylene exposure and therefore functional CNS disturbance is regarded as a key endpoint in humans, although the available data do not allow conclusive judgements to be made regarding causal or dose-response relationships. Notwithstanding the dose-response uncertainties, the overall view reached by the majority of Member States in discussing this endpoint is that there appears to be an absence of CNS effects associated with exposure levels of around 50 ppm (supported by Rasmussen et al., 1993; Landrigan and Kominsky et al., 1988), so this value was identified as a NOAEL for functional CNS disturbance in humans.

Kidney toxicity, the most sensitive endpoint in animal models, has not been properly investigated in humans.

Intolerance to alcohol, presenting as a transient redness affecting mainly the face and neck (generally known as “trichloroethylene or degreasers' flush”) has also been frequently observed in exposed workers. Identical effects have been seen in single and repeated dose volunteer studies, confirming that this effect is caused by trichloroethylene.

Some studies included an investigation for possible liver effects. Evidence of the presence of liver damage in trichloroethylene-exposed workers was seen in certain studies, seen as by hepatomegaly or changes in blood clinical chemistry parameters. However, such findings were not seen other studies, including one involving workers who had experienced relatively high exposure levels. Overall, there was no consistent, convincing evidence of trichloroethylene-related liver damage.

The potential neurotoxicity of trichloroethylene has investigated using specific tests in limited number of studies involving occupational cohorts. The available studies provided no evidence of significant effects on nerve conduction velocity of EEG patterns. Effects on performance in neuropsychological and reaction-time tests have been reported in two studies, but the data were too limited to draw conclusions about their relationship with trichloroethylene exposure. A number of studies have also been carried out to investigate the effect of occupational exposure to trichloroethylene on the cardiovascular system, particularly cardiac rhythm. The value of these is limited both by the lack of data on atmospheric trichloroethylene concentrations and the failure to use an adequately matched control group. Some very limited evidence is available to suggest that exposure to about 100 ppm of trichloroethylene may produce ventricular arrhythmias in a small proportion of the persons exposed. However, the number of subjects affected was very

low, and further work is needed to investigate this aspect before any definitive conclusions can be drawn.

There are occasional case reports describing patients who had been exposed to trichloroethylene at work with conditions such as Stevens-Johnson syndrome or scleroderma. It is possible that these may represent rare idiosyncratic reactions to trichloroethylene, but with the available information it is not possible to draw any conclusions about causality.

Overall, taking a precautionary stance in view of uncertainties regarding causal and dose-response relationships, functional CNS disturbance is considered to be the most sensitive endpoint in humans. The majority of Member States considered that a NOAEL of 50 ppm could be identified from the available human data. This value is therefore taken forward to the risk characterisation for repeated exposure. However, it is noted that in animal studies, a clear NOAEL for neurotoxicity of 200 ppm has been identified for long-term inhalation exposure to trichloroethylene. In view of this, and given the uncertainties surrounding the identification of a reliable NOAEL for humans from the available data, it is possible that actual NOAEL for neurotoxicity in humans may be higher than 50 ppm.

In animals, kidney toxicity appears to be the most sensitive endpoint for both long-term repeated inhalation and oral exposure. Comparing the human and animal data, there are no reports of trichloroethylene-related kidney toxicity in the human studies, but this endpoint has not been properly investigated in humans. Therefore, because of the paucity of human data for this endpoint, the animal data must be considered in the risk characterisation. The NOAEL of 100 ppm will be taken forward to the risk characterisation.

4.1.2.7 Mutagenicity

4.1.2.7.1 *In vitro* studies

The mutagenicity of trichloroethylene has been extensively investigated in a number of *in vitro* test systems. Some tests used trichloroethylene which contained the epoxide stabilisers epichlorohydrin and/or 1,2-epoxybutane. These epoxides are known mutagens and consequently positive tests involving epoxide-stabilised trichloroethylene do not provide any information on the potential mutagenicity of pure trichloroethylene.

Bacterial systems

Trichloroethylene, either with or without epoxide stabilisers, has been extensively tested in bacterial (Ames) systems. Exposures have involved either the vapour (plates were exposed in a desiccator, which prevented vapour loss) or liquid phase.

Vapour phase

Crebelli et al. (1982), in a well-conducted study, investigated the mutagenicity of epoxide-free and epoxide-stabilised (containing 0.19% 1,2-epoxybutane and 0.09% epichlorohydrin) trichloroethylene vapour using *Salmonella typhimurium* strain TA 100. Epoxide-stabilised trichloroethylene produced a clear, reproducible, positive response, both in the presence and absence of an exogenous metabolising system (Aroclor-induced rat liver S9). For epoxide-free trichloroethylene (confirmed by analysis) there was no increase in the numbers of revertants/plate in the absence of S9. However, in the presence of S9, epoxide-free

trichloroethylene elicited a dose-related and statistically significant increase in the number of revertants/plate. This increase (between 1.5 and 2 fold at the highest concentration), although less than that seen in the positive control and stabilised trichloroethylene exposed plates, was reproducible in nine experiments, involving the use of three different Aroclor-induced rat liver S9 preparations, and is therefore considered to represent a positive response.

In another study involving exposure to the vapour, epoxide-stabilised (containing 0.5% 1,2-epoxybutane) and "low-stabilised" trichloroethylene were tested using *S. typhimurium* strains TA100 and TA1535 (Shimada et al., 1985). Toxicity was seen at the highest concentrations. Epoxide-stabilised trichloroethylene elicited a positive response in both strains in the presence or absence of Aroclor-induced rat liver S9. The low-stabilised sample tested negative. The results of this study were not apparently confirmed by independent experiment.

McGregor et al. (1989), in a well-conducted study, tested the mutagenic potential of epoxide-free trichloroethylene (Hi-Tri® grade, containing an amine stabiliser) vapours at non-toxic concentrations of up to 20% in *S. typhimurium* strains TA 98 and TA 100 in the presence of Aroclor-induced rat or hamster liver S9. No evidence of mutagenic activity was observed. However, epoxide-stabilised trichloroethylene (containing 0.5% 1,2-epoxybutane) vapour tested positive in strains TA 1535 and TA 100 at concentrations of 0.63% and above. The presence or absence of rat liver S9 had little influence on the observed response. The commonly used epoxide stabilisers were also tested, in TA 1535 and TA 100 in the absence of S9, and clear evidence of mutagenic activity was reported for 1,2-epoxybutane vapour at a concentration of 0.009% and for epichlorohydrin vapour at 0.0009%.

In an earlier study, epoxide-free trichloroethylene vapour elicited an increase in the numbers of revertants (mean 135 revertants/plate at a concentration of 5% compared with 75/plate in unexposed plates) in *S. typhimurium* strain TA 100 in the presence of mouse liver S9 (Bartsch et al., 1979). The reproducibility of this relatively small increase was not investigated. In another study, epoxide-free trichloroethylene vapour produced a statistically significant and reproducible increase in the number of revertants in TA 100 in the presence of S9 at concentrations of 1 and 3%. However, the increase was not dose-dependent and was only about 30% above the background rate (Baden et al., 1979). These two studies are considered not to provide clear evidence of genotoxic activity.

Several vapour phase studies have been reported in which the presence or absence of epoxide stabilisers in the trichloroethylene test sample was not described. No evidence of mutagenic activity was observed in the presence of rat liver S9 in *S. typhimurium* strains TA 100 and 98, at concentrations up to 10% (Waskell, 1978). In three very briefly reported studies, trichloroethylene vapour produced responses which were considered by the authors to represent evidence of mutagenic activity, although it was not possible to verify the authors' conclusions because the data were not presented (Milman et al., 1988; Warner et al., 1988; Riccio et al., 1983).

Liquid phase

Trichloroethylene (>99.9% purity) produced no evidence of mutagenic activity in *S. typhimurium* strains TA 98, 100, 1535 and 1537, in the presence or absence of exogenous metabolising systems (Aroclor induced rat or hamster liver S9); toxicity was seen at the highest concentration tested (Mortelmans et al., 1986). Similarly, in another study epoxide-free trichloroethylene was negative in TA 100, although it is not clear if toxic concentrations of the test substance were included in the assay (Henschler et al., 1977).

McGregor et al. (1989) investigated the mutagenic potential of epoxide-stabilised trichloroethylene (containing 0.5% 1,2-epoxybutane) in *S. typhimurium* strains TA 98 and 100, in the presence or absence of rat liver S9. Concentrations of up to 10 mg/plate were tested, and no evidence of mutagenic activity was observed. Several other mutagenicity tests have been published, describing positive (Cerna and Kypenova, 1977; Hughes et al., 1987) or negative (Calandra et al., 1987) results, but these contain no information on the composition of the trichloroethylene tested and are very briefly reported, making it impossible to judge the acceptability of the test method or validate the conclusions of the authors.

Greim et al. (1975) investigated the mutagenicity of trichloroethylene (probably of analytical grade) in *Escherichia coli* (K12 strain) in a study involving a single exposure concentration. Gal+, arg+ and nad+ reverse mutation systems and a resistance to 5-methyl-DL-tryptophane forward mutation system were used. An increase in the numbers of mutant colonies, only in the presence of an exogenous metabolising system, was described for the gal+ (20% increase in background rate), arg+ (132 % increase) and MTR (14% increase) systems. It is difficult to assess the significance of this finding because a non-standard test system was used.

The mutagenic potential of trichloroethylene in bacteria (*S. typhimurium* strain TA 98) has also been investigated in a host-mediated assay (NIOSH, 1980). Groups of five male and five female mice were exposed to trichloroethylene (purity 99.9%) by the inhalation route for five days at concentrations of 100 or 500 ppm. A suspension containing 1010 bacterial cells was then injected via the intraperitoneal route. Cells were recovered three hours later in intraperitoneal fluid and the numbers of revertants/ml were determined. Negative (filtered air) and positive (2-amino-anthracene by the intraperitoneal route) control groups were included and the experiment was repeated. No clear evidence of mutagenic activity was observed in the trichloroethylene treated groups. However, an appropriate response was not observed in the positive control groups and it is therefore not possible to draw any conclusions about the mutagenic potential of trichloroethylene on the basis of this study.

Because of the possibility that DCVC metabolites may have a role in trichloroethylene induces mutagenesis and carcinogenesis, bacterial mutagenicity assays of these metabolites are briefly considered. S-(1,2-dichlorovinyl)-L-cysteine tested positive in *S. typhimurium* strain TA 100 in the presence or absence of rat kidney S9 (Dekant et al., 1986b; Green and Odum, 1985). In S. typhimurium strain TA2638, 2,2-dichlorocysteine and its mercapturic acid were mutagenic, but with less potency than the corresponding 1,2-isomers (Commandeur et al., 1991). Vamvakas et al. (1987) showed that the mutagenicity of both 1,2-dichlorocysteine and its mercapturic acid could be inhibited by the inhibition of bacterial β-lyase.

To summarise the results of bacterial mutagenicity tests, epoxide-stabilised trichloroethylene vapour was consistently positive, both in the presence or absence of an external metabolising system. Epoxide-free trichloroethylene vapour gave mixed results, but the Crebelli et al. (1982) study provides clear evidence of mutagenicity, requiring the presence of an exogenous metabolising system, which cannot be overruled by negative results in other studies. Tests using liquid trichloroethylene, either with or without epoxide stabilisers, were generally negative. The mutagenicity of the glutathione metabolites of trichloroethylene has been demonstrated.

Fungal systems

Both non-stabilised and epoxide stabilised (containing epichlorohydrin and 1,2 epoxybutane) liquid phase trichloroethylene showed no evidence of mutagenic activity when tested up to toxic concentrations, both in the presence and absence of an exogenous metabolising system (β-naphthoflavone or phenobarbital induced rat or mouse liver S9) in the yeast

Schizosaccharomyces plombe, strain P1 (Rossi et al., 1983). Epichlorohydrin and 1,2-epoxybutane were also tested in this system and were found to be positive, both in the presence and absence of induced liver S9. A host-mediated assay was also conducted, in which mice (group size 4-8) were injected by either the intrasanguinous or intraperitoneal route with the yeast cells following oral administration of either non-stabilised or epoxide-stabilised trichloroethylene at a dose level of 2,000 mg/kg bodyweight. Again, no evidence of mutagenic activity was detected.

In a recent study in *Saccharomyces cerevisiae* strain D61.M, liquid phase trichloroethylene (purity 99%, presence or absence of stabilisers was not reported) caused increased numbers of white cycloheximide-resistant auxotrophic colonies, but further investigation indicated that these may have arisen as a result of the induction of "respiratory deficiency" rather than from chromosome loss (Whittaker et al., 1990). In an earlier study, the potential mutagenicity of analytical grade trichloroethylene was investigated in *S. cerevisiae* strains D7 and D61.M, both in the presence and absence of Aroclor induced mouse liver S9 (Koch et al., 1988). No evidence of mutagenic activity was seen with strain D7, when exposed to moderately toxic concentrations of trichloroethylene. However, in the assay with strain D61.M, an increased number of white, cycloheximide-resistant and leucine-requiring colonies were observed, considered by the authors to indicate the induction of aneuploidy. The response was dose-related and was not influenced by the presence of S9. The possibility that these colonies may have arisen as a result of respiratory deficiency was not investigated, which may weaken the validity of the author's conclusion.

The mutagenicity of liquid phase technical grade trichloroethylene (no information was given on the presence of stabilisers) was investigated in *S. cerevisiae,* strain XV 185-14C (Shahin and von Borstel, 1977). No evidence of mutagenicity was observed in the absence of an exogenous metabolising system. However, in the presence of mouse liver S9 there was a marked increase in the numbers of revertants, although this was seen only at concentrations that were very toxic to the yeast cells (survival was < 1%); no conclusions can be drawn from such results.

In another assay using *S. cerevisiae* (strain D7)*,* conducted without an exogenous metabolising system, evidence of mutagenic activity was seen following exposure to liquid phase trichloroethylene of unspecified purity at a concentration which elicited moderate cytotoxicity (Callen et al., 1980). The frequencies of gene conversion at the trp5 locus, mitotic recombination at the ade2 locus and gene conversion at the ilv locus were all increased (between 2- and 14-fold) in comparison with a negative control group.

Bronzetti et al. (1978) tested liquid phase trichloroethylene of unspecified purity in *Saccharomyces cerevisiae*, strains D4 and D7. The range of concentrations tested included toxic levels; survival was about 50% at the highest concentration. In the presence of mouse liver S9 there was a reproducible and dose-related increase in the numbers of revertants and convertants. No evidence of mutagenic activity was seen in the absence of S9. A host-mediated assay was also conducted, involving the intrasanguinous instillation of yeast cultures ($2 \cdot 10^9$ cells) to groups (size 3-4) of mice which had been treated on the day of instillation with a single oral dose of trichloroethylene at 400 mg/kg. Further groups also received additional repeated oral exposure at a level of 150 mg/kg/day (twenty-two administrations over a four week period) prior to instillation. Increased numbers of mutants were observed in cultures recovered from the liver and kidneys, but not from the lungs.

Crebelli et al. (1985) investigated the ability of epoxide-free trichloroethylene to induce gene mutations and mitotic segregation in *Aspergillus nidulans*, strains 35 and 35x17, and reported positive results. This assay has not been internationally validated, which limits the significance that can be given to this result.

To summarise the fungal test data, trichloroethylene gave conflicting results. All the positive tests used trichloroethylene of unspecified purity, so it is possible that these tests demonstrated the presence of mutagenic activity that was related to epoxide stabilisers. Overall, because of the inconsistencies, it is not possible to draw any firm conclusions about the mutagenicity of trichloroethylene on the basis of these studies.

Mammalian cells

In a well-conducted mouse lymphoma L5178Y/tk gene mutation test, epoxide-free liquid phase trichloroethylene (Hi-Tri® grade, containing an amine stabiliser) elicited a positive response in the presence of Aroclor induced rat liver S9 (NTP, 1988). A reproducible doubling compared to the negative control mutation frequency was seen at the highest concentration tested. The assay was negative in the absence of S9. Caspary et al. (1988) and Myhr and Casparay (1991) also present positive trichloroethylene mouse lymphoma assays, but these are probably both further reports of the experiment described by NTP (1988). Another report of a positive (only in the presence of S9) mouse lymphoma test is available, but only as an abstract so it is not possible to verify the authors' conclusions (Rudd et al., 1983).

In a well-conducted chromosome aberration test in Chinese hamster ovary (CHO) cells, epoxide-free trichloroethylene (Hi-Tri® grade, containing an amine stabiliser) was negative (NTP, 1988; Galloway et al., 1987). The presence or absence of toxicity was not reported, but the highest concentration used was 14.9 mg/ml, which is in excess of the usual maximum dose level of 5 mg/ml.

In a sister chromatid exchange (SCE) assay using CHO cells, trichloroethylene (purity unspecified) was negative (White et al., 1979). However, the test did not appear to involve exposures at levels that produced toxicity, which limits its value. In another CHO cell SCE assay, the results were equivocal (Galloway et al. 1987). Both in the presence and absence of rat liver S9, the frequency of SCE at the highest concentration was significantly greater than the negative controls and a dose-response relationship was evident. However, the frequencies of SCEs in the exposed cells were within the background range for negative control cultures, casting doubts as to whether this should be considered a positive result.

The potential for epoxide-stabilised (containing 0.5% 1,2-epoxybutane) and “low-stabilised” trichloroethylene, as either vapour or liquid phase, to induce unscheduled DNA synthesis (UDS) in rat hepatocytes has been investigated (Shimada et al., 1985). Autoradiography was used to assess UDS. The assay was conducted at cytotoxic concentrations. The results were negative for all the forms of trichloroethylene. In contrast, positive results have been described in other UDS assays, which all used scintillation counting techniques to measure UDS. NIOSH (1980) tested trichloroethylene (99.9% purity) in human WI-38 cells and reported a response, both in the presence and absence of rat liver S9, which was considered positive according to the laboratory's criteria. However, a dose-response relationship was not evident and an appropriate response was not observed in cells exposed to the positive control substance in the presence of S9, casting doubts regarding the validity of this assay. Costa and Ivanetich (1984) found that trichloroethylene induced UDS in hepatocytes derived from phenobarbital treated rats, and Perocco and Prodi (1981) reported similar findings in human lymphocytes; for both studies the purity of the trichloroethylene was not defined and in the former study it was not clear that an adequate number (at least two) of replicates was used.

To summarise the *in vitro* mammalian cell data, epoxide-free trichloroethylene was positive in a mouse lymphoma gene mutation assay, requiring the presence of an exogenous metabolising system. The genotoxicity of trichloroethylene has also been examined in chromosome aberration,

SCE and UDS assays; however, it was not possible to draw firm conclusions from these data because the results were inconsistent and most tests had limitations. Overall, there is clear evidence that trichloroethylene can induce mutations in mammalian cells.

Summary of *in vitro* studies

The genotoxicity of trichloroethylene has been investigated in bacterial, fungal and mammalian tests. Two well-conducted assays which used standard methods, namely a bacterial (Ames) test and a mouse lymphoma gene mutation assay, provide convincing evidence that epoxide-free trichloroethylene is an *in vitro* mutagen. The provision of an exogenous metabolic activation system was required for expression of this activity.

4.1.2.7.2 Drosophila

Epoxide-free trichloroethylene (purity 99.9%) was negative in a *Drosophila melanogaster* sex-linked recessive lethal test (NIOSH, 1980). However, it is not clear whether or not the study was conducted at exposure levels that produced toxicity and the result did not appear to have been confirmed in a separate experiment.

4.1.2.7.3 *In vivo* tests

Somatic cells

Micronucleus tests

Trichloroethylene (unspecified purity) was unequivocally negative in a well-conducted mouse micronucleus test (male, B6C3F1 strain) involving intraperitoneal administration (Shelby et al., 1993). Groups of four to six mice received three consecutive daily injections at levels of 0, 500, 1,000 and 2,000 mg/kg and were killed 24 hours after the last injection. There were no increases in the micronucleated polychromatic erythrocytes (PCEs) among treated animals. The test was repeated using dose levels of 0, 2,000 and 2,500 mg/kg and again the results were considered to be negative. Evidence of general toxicity (mortality) was apparent at 2,000 and 2,500 mg/kg. Considerable weight is given to this negative result because the study was conducted according to internationally recognised guidelines, employing dose levels and a route of administration that ensured that exposure of the target tissue to the test substance (and its metabolites) was maximised.

Several other micronucleus tests have been published, but all present interpretation difficulties. In the most recent study, groups of five male rats or C57BL/6J mice (five males per group) received a single 6-hour exposure to reagent grade trichloroethylene (purity >99%) by the inhalation route at nominal concentrations of 0, 5, 500 or 5,000 ppm (Kligerman et al., 1994). Additionally, groups of five male rats received four consecutive daily 6-hour exposures at nominal concentrations of 0, 5, 50 or 500 ppm. The animals were killed 18 hours after termination of exposure and the presence of micronuclei in binucleated peripheral blood leukocytes (PBLs) (rats only) or splenocytes (mice only) and bone marrow PCEs was assessed. Chromosome aberration and SCE examinations were also conducted in these animals (see below). Among the rats given a single trichloroethylene exposure there was a statistically significant and dose-related increase in the numbers of micronucleated PCEs; at the highest exposure level the increase was about four-fold in comparison with the controls (6.6 ± 0.4 (mean

± SD) micronucleated PCEs/1,000 at 5,000 ppm, 1.7 ± 0.8 for the control group). A subsequent replication of the 5,000 ppm exposure, conducted with two negative control groups, demonstrated that the effect was reproducible (frequency of 5.8 ± 1.6 micronucleated PCEs/1,000 at 5,000 ppm, compared with 1.9 ± 0.2 and 2.1 ± 0.8 for the controls). In contrast, trichloroethylene had no effect in rats on the numbers of micronucleated PCEs in the groups with repeated exposure or on the numbers of micronucleated PBLs following either a single or repeated exposure. Similarly, trichloroethylene had no effect on the numbers of micronuclei in mice splenocytes or PCEs. At first sight, the results for the rats receiving a single exposure appear to provide clear evidence of trichloroethylene-related genotoxic activity. However, this issue is clouded by the observation among rats of the repeated exposure control group, that the frequency of micronucleated PCEs was relatively high and variable (mean 4.0 ± 1.9 micronucleated PCEs/1,000, with a highest individual frequency of 6.5) and also comparable with the frequency reported in the groups receiving a single exposure to trichloroethylene. This suggests that the increased frequency of micronuclei in the trichloroethylene single exposure groups could possibly have arisen by chance alone. The idea that these changes should not be interpreted as a positive result receives some support from the absence of evidence of mutagenic activity in the chromosome aberration and SCE investigations conducted in the same animals (see below). Overall, the results of this study are considered to be equivocal.

Duprat and Gradiski, (1980) administered analytical grade trichloroethylene (purity 99.5%) by the oral route to groups of 10 CD1 strain mice at levels of 0, 375, 750, 1,125, 2,250 and 3,000 mg/kg. Two single doses, separated by 24 hours were given, and the animals were killed 16 hours later. A dose related increase in the percentage of micronucleated PCEs was observed. At the highest treatment level the percentage was approximately 16-fold that of a vehicle control group. However, based on the authors' description of the scoring method ("micronuclei, and microbodies appearing to be of nuclear origin, were observed") and because the frequency of micronucleated PCEs in the control groups was unusually high (10/1,000), it is possible that bodies other than micronuclei were scored. The uncertainty relating to the scoring method limits the significance that can be attached to this apparently positive result.

A positive result was also reported in another micronucleus study involving the administration of a single oral dose of epoxide-free trichloroethylene to B6C3F1 mice at a level of 1,200 mg/kg (Sbrana et al., 1985, cited in ECETOC, 1994). The study report is only available as an abstract and consequently it is not possible to assess quality of the study or the validity of the authors' conclusions.

Sujatha and Hegde (1998) investigated micronucleus formation in Swiss albino mice, but because of reporting inconsistencies, the reliability of this study is called into question (see below for further description of this study)

In a study using non-standard methodology, the ability of trichloroethylene to induce micronuclei in the rat kidney was investigated (Robbiano et al., 1998). Trichloroethylene (purity 99.5%) was administered as a single dose of 525 mmol/kg to a group of seven Sprague-Dawley rats. Negative and positive (NDMA) control groups were included. Prior to treatment, the rats had the left kidney removed and were administered folic acid (250 mg/kg, oral) to stimulate proliferative activity in the cells of the remaining kidney. The rats were killed 2 days after experimental treatment. Kidney cells were removed and isolated by trypsin and collagenase and prepared for microscopic examination. The frequencies of micronucleated and binucleated cells were determined by scoring at least 2,000 cells per rat. The mean (± SD) frequency of micronucleated cells in the trichloroethylene treated group was 4.3 $(\pm 1.2) \cdot 10^{-3}$, compared with 1.33 $(\pm 0.4) \cdot 10^{-3}$ for the negative control group. The frequency in the positive control group was

$8.7 (\pm 2.7) \cdot 10^{-3}$. The frequency of binucleated cells, considered by the authors to be a measure of cytotoxicity, was decreased in the trichloroethylene group by about 30% relative to the negative control. The report does not specify if the type of micronuclei produced contain whole chromosomes or chromosome fragments. This information would help to interpret the results because whole chromosome loss could be related to disturbances in chromosome segregation resulting from the stimulated proliferation. The presence of binucleated cells in both the control and treated cells provides evidence that forced proliferation may have disturbed cell cycle progression.

Tests for chromosome aberrations and sister chromatid exchange

Kligerman et al. (1994), as part of the previously described inhalation study, conducted chromosome aberration and SCE analyses in rat PBLs and mouse splenocytes in parallel to the previously described micronucleus analysis. No changes were reported in the trichloroethylene exposed rats or mice.

The potential for trichloroethylene to induce chromosome aberrations following inhalation exposure has also been investigated in an earlier study (NIOSH, 1980). Groups of ten male and ten female Sprague-Dawley rats received either single 7-hour exposure or five consecutive daily 7-hour exposures at levels of 0, 100 or 500 ppm. Bone marrow cells were sampled from the animals receiving a single exposure at 6, 24 or 48 hours after exposure and from the groups with repeated exposure at 6 hours after the last exposure. Trichloroethylene had no effect on the frequency of chromosome aberrations. A limitation of this study is that it is not clear if the study was conducted at concentrations which elicited generalised toxicity.

Other negative *in vivo* chromosome aberration tests have been reported, using the oral (Loprieno and Abbondandolo, 1980; Sbrana et al., 1985 (cited in ECETOC, 1994)) and intraperitoneal (Cerna and Kypenova, 1977) routes. These tests were only briefly reported and consequently a critical review was not possible.

The possible aneugenic effects of trichloroethylene were investigated in Swiss albino mice (Sujatha and Hegde, 1998). The presence of C-mitotic effects, micronuclei and chromosome aberrations were investigated. Groups of 5 mice per endpoint received a single intraperitoneal dose of trichloroethylene (purity 99%) of 500, 1,000, 2,000 or 4,000 mg/kg. Vehicle (DMSO), water and positive (cyclophosphamide (25 mg/kg) groups were included. The animals were killed 6, 12, 24 and 48 hours after dosing and bone marrow was sampled for the three investigations. C-mitoses were increased in the 2,000 and 4,000 mg/kg groups, seen in parallel with an increased mitotic index and decreased anaphase frequency. There was an apparent substantial increase in the numbers of chromosome aberrations among the trichloroethylene treated groups according to the tabulated results, but these were inexplicably not declared statistically significant and the text did not draw attention to these increases. There were also inconsistencies between the tabulated results and text explanation for the micronuclei data; the table showed statistically significant increases for micronucleus frequency at 2,000 and 4,000 mg/kg, in comparison with vehicle controls, but these were not flagged in the text. Because of these inconsistencies the reliability of the study must be called into question.

Transgenic mouse model

No evidence of trichloroethylene-induced mutagenicity was observed in an inhalation study using the lacZ (Muta™ mouse) model (Douglas et al., 1995; 1999). Groups of between 5 and 10 males were exposed to trichloroethylene (purity 99+%) at concentrations of 0, 200, 1,000 and 3,000 ppm, 6 hours/day for 12 days. The highest concentration was described as being the

maximum tolerated dose. Trichloroethylene was found not to have induced mutations of the lacZ gene in liver, lung and bone marrow, sampled 14 and 60 days after termination of exposure. This assay has not, at present, been fully validated, and consequently the negative result cannot be regarded as definitive, although it provides evidence in support of a view that trichloroethylene is not an *in vivo* mutagen.

Mouse spot test

In a mouse spot test, a single dose of trichloroethylene (purity 99.5%) was administered by the intraperitoneal route at levels of 140 or 350 mg/kg on day 11 of pregnancy to females of the C57BL/6JHan strain which had been mated with T-stock males (Fahrig, 1977). The numbers of offspring with spots of a type that are presumed to have resulted from somatic mutation was 2/145 at the lower dose level and 2/51 at the higher level. For a pooled negative control group, 1/794 offspring had genetically relevant spots. It is not at all clear if this pattern of results can be regarded as a positive outcome.

In a modified mouse spot test, the potential of trichloroethylene to induce reversion events in embryonic melanocytes has been investigated in mice homozygous for the pink-eyed dilution unstable mutation (C57BL/6Jp^{un}/p^{un}) (Schiestl et al. 1997). Trichloroethylene (described as "highest purity grade") in corn oil was administered to a group of 18 mated female mice as an intraperitoneal injection at 200 mg/kg on day 0.5 post conception. Reversion events can be seen as black spots on the grey coat of the offspring, which were examined for at days 12-14 of age. In the trichloroethylene treated group, 32% of offspring had black spots, compared with a frequency of 4-11% in negative control groups. The mothers were in a state of sedation for several hours after treatment. Litter size was reduced by more than 50% in the trichloroethylene group. This study is difficult to interpret in terms of the potential genotoxicity of trichloroethylene because it is not a widely used standard assay (probably this assay has only been conducted in the author's laboratory). No rationale was given for the choice of dose level, which caused a high level of embryolethality, or for the route of administration. In view of the high level of embryotoxicity the possible influence of cytotoxicity in the observed changes is unclear.

Tests for UDS, DNA binding and damage

Mirsalis et al. (1989) investigated the ability of trichloroethylene (of unspecified purity) to induce UDS in Fischer 344 rats and B6C3F1 mice. Groups of three rats or mice received three (presumably on consecutive days) oral doses of trichloroethylene at levels of 50, 200 and 1,000 mg/kg; larger numbers of animals were allocated to positive and negative control groups. Autoradiography was used to measure UDS in primary hepatocyte cultures prepared from animals killed at 2 or 12 hours after treatment. Trichloroethylene did not induce UDS in rat or mouse hepatocytes. However, there was evidence of hepatocellular proliferation, observed as an increased percentage of cells in S-phase; this effect was more marked in males than females. Negative results were also reported in a similar UDS assay in mice (Doolittle et al., 1987).

Stott et al. (1982) found little evidence of hepatic DNA alkylation in a group of four mice, killed 5 hours after a single oral dose of [^{14}C]trichloroethylene at a level of 1200 mg/kg. Parchman and Magee (1982) also conducted *in vivo* DNA binding studies and found no clear evidence of trichloroethylene binding in the liver of rats following administration of single intraperitoneal doses of between 10 and 1,000 mg/kg. DNA fragmentation studies were also conducted by Parchmann and Magee (1982). They found that a 2,000 mg/kg intraperitoneal dose of

trichloroethylene (purity >99%) had no effect on DNA fragmentation in mouse liver, measured by alkaline sucrose gradient centrifugation.

Nelson and Bull (1988) studied the induction of single strand breaks (SSBs) in DNA in the liver following the oral administration of single doses of epichlorohydrin-free trichloroethylene to male Sprague-Dawley rats and male B6C3F1 mice. SSBs were measured using an alkaline unwinding assay. Doses of 3 to 4 mg/kg (22 to 30 mmol/kg) in the rat and 1.5 mg/kg (11.4 mmol/kg) in the mouse caused the induction of SSBs. Pre-treatment of rats with trichloroethylene or phenobarbital reduced the dose of trichloroethylene required to induce SSBs. In another study using similar techniques, the induction of SSBs was found in the liver and kidneys but not lungs of mice following the intraperitoneal administration of trichloroethylene (purity 99.5%) (Walles, 1986). The biological significance of such effects is uncertain.

The effects of trichloroethylene and its metabolite S-1,2-dichlorovinylcysteine (DCVC) on kidney cell DNA were investigated in a comet assay (CTL 1998). Comet formation was assessed in kidney proximal tubule cells from groups of five rats exposed to trichloroethylene (purity 99.5%) by inhalation or DCVC by the oral route. Inhalation exposures were by whole body to airborne trichloroethylene concentrations of 0, 500, 1,000 or 2,000 ppm, 6 hr/day for five days and the animals were killed immediately after the fifth exposure. DCVC was administered as a single dose of 0, 1 or 10 mg/kg, and the animals killed at 2 and 16 hours after dosing. A concurrent *in vitro* positive control group involved exposure of kidney cells taken from the oral negative control group animals and exposing *in vitro* to N-methyl-N'-nitro-N-nitrosoguanidine at a concentration of 5 μg/ml. A supplementary study was conducted in which groups of rats received DCVC as a single oral dose of 10 mg/kg and an *in vivo* positive control group received N-nitrosodimethylamine as a single dose of 20 mg/kg; animals were killed at 2 and 16 hours after dosing. Slides of isolated kidney proximal tubule cells were prepared by gel electrophoresis for comet analysis. One hundred and fifty cells per animal were analysed. In comparison with the negative control groups there were no differences considered to be treatment related in tail length, % tail DNA content or tail moments for any of the groups receiving trichloroethylene or DCVC groups. For the *in vitro* and *in vivo* positive control groups, tail length, % tail DNA content and tail moment were markedly increased in comparison with the negative control group.

Germ cells

The mutagenic activity of trichloroethylene in germ cells has been investigated using the dominant lethal assay. In one study, groups of 50 male mice (NMRI-Han/BGA strain) received a single 24-hour inhalation exposure to trichloroethylene (purity 99.5%, epoxide free) at concentrations of 0, 50, 200 and 450 ppm (Slacik-Erben et al., 1980). Sequential mating trials were conducted with untreated females in which the females were exchanged every four days, altogether twelve times. No evidence of the induction of dominant lethal mutations was observed. However, this study did not involve exposure to toxic concentrations of trichloroethylene, and consequently the significance that can be attached to this negative result is limited. Another inhalation dominant lethal study is available, using rats, but it is not possible to interpret the results of the study due to high frequencies of dead implantations among females of the negative control group (NIOSH, 1980).

The ability of trichloroethylene to induce micronuclei in mouse spermatids has been investigated (Allen et al., 1994). Groups of six C57B1/6J strain mice were exposed to trichloroethylene (purity $\geq$ 99%) by the inhalation route at concentrations of 0, 5, 50 or 500 ppm, for 6 hours/day for 5 consecutive days. The frequency of micronuclei in spermatids harvested 14 days after the

initial exposure was not affected by treatment. No information on the presence or absence of general toxicity was presented, which raises a question mark against the appropriateness of the chosen exposure concentrations.

Summary of *in vivo* data

The *in vivo* genotoxicity of trichloroethylene has been extensively investigated in somatic cells. Overall, it is concluded on a weight of evidence basis, that trichloroethylene is not an *in vivo* mutagen, provided by clear negative results in well-conducted standard micronucleus (Shelby et al., 1993), chromosome aberration and SCE (Kligerman et al., 1994) and UDS (Mirsalis et al., 1989) assays, with support from negative results the non-validated Comet (CTL, 1998) and transgenic Muta™ mouse (Douglas et al.,1999) assays. There were some limited data that do not support this conclusion, namely a micronucleus test with results that were considered equivocal (Kligerman et al., 1994), a further micronucleus test of questionable validity which was reported as positive (Duprat and Gradiski, 1980), and two non-standard tests of uncertain toxicological significance (Robbiano et al., 1998; Schiestl et al., 1997). However, this evidence is considered to be of insufficient strength to overturn the conclusion that trichloroethylene is not an *in vivo* mutagen. The potential for trichloroethylene to induce germ cell mutations has not been extensively investigated, but the available evidence indicates that trichloroethylene does not possess this activity.

4.1.2.7.4 Human genotoxicity

The investigation of possible trichloroethylene-related genotoxic activity in humans has been investigated in several studies. Three have looked for SCE induction, one examined clastogenicity and, most recently, two studies have investigated the presence of mutations in the von Hippel-Lindau (VHL) tumour suppressor gene in renal cell cancer patients with occupational exposure to trichloroethylene.

Gu et al. (1981) compared the frequency of SCE in peripheral blood lymphocytes of a group of six exposed workers with nine, apparently unmatched, controls. The frequency of SCE was slightly greater in the exposed group, but no conclusions could be drawn from this study because of a number of study design deficiencies, such as small group size, poor characterisation of exposure and lack of consideration of potential confounding factors such as smoking.

In a better designed study, although again limited by small group sizes, Nagaya et al. (1989), compared SCE frequencies in twenty-two trichloroethylene workers with an identical number of control subjects, matched with respect to age, sex and smoking habits. Total “trichloro compound” concentration in the urine was measured, which indicated, according to the authors, that the trichloroethylene workers were exposed on average to atmospheric concentrations of about 30 ppm trichloroethylene. There were no differences between the exposed and control groups in the frequency of SCE.

Seiji et al. (1990) analysed the frequency of SCEs in a small group of trichloroethylene workers by sex and by smoking habits. Breathing zone trichloroethylene concentrations were described as being between 10 and 50 ppm. Among male smokers, the frequency of SCEs in exposed workers (7.1 ± 1.4 SCEs/cell, group size 8) was statistically significantly greater than age-matched controls (5.1 ± 1.2 SCEs/cell, group size 7). Among females and male non-smokers, there were no differences between the control and exposed groups. However, because of the small group sizes no conclusions can be drawn from this study.

Rasmussen et al. (1988) investigated the frequency of sperm with two fluorescent bodies (thought to indicate the presence of two Y-chromosomes) and frequency of chromosome aberrations in lymphocytes among a small group of trichloroethylene workers. No exposure data were presented. Sperm analysis was conducted on twelve exposed workers and fourteen apparently non-matched controls. There were no clear differences between the two groups. However, in the chromosome aberration investigation it was found that the number of metaphases with gaps among fifteen exposed workers was significantly greater in comparison with a control group of 669 individuals. The increased incidence among the exposed workers was due primarily to increases in three individual workers that were described as having the highest exposures, based on urinary trichloroethylene values (which were not presented). Unfortunately, the possible confounding influence of smoking did not appear to have been taken into consideration. Because of this limitation, and mindful of the small numbers of exposed workers investigated, it is considered that no firm conclusions can be drawn from this study. Furthermore, it is not clear that the presence of gaps in chromosome aberration studies is of biological significance.

Twenty-three patients with histologically verified renal cell carcinoma and with a history of occupational exposure to trichloroethylene were analysed for the presence of somatic mutations within the VHL tumour suppressor gene (Brüning et al., 1997). These patients were apparently also subjects in the Vamvakas et al. (1998) case control study (see carcinogenicity section), and were actively seeking compensation for claimed trichloroethylene-related occupational disease. DNA was isolated from tumour cells, amplified by polymerase chain reaction and analysed in single strand conformation polymorphism and sequencing. All 23 patients were found to have aberrations in the VHL gene; for 30% these were in exon 1, 44% in exon 2 and 26% in exon 3.

In a further study by the same team, the presence of VHL mutations was investigated in 44 renal cell cancer patients who had a history of occupational exposure to trichloroethylene (Brauch et al., 1999). These patients were actively seeking compensation for claimed trichloroethylene-related occupational disease. Based on trichloroethylene-related clinical symptoms reported by the patients, employment history and knowledge of the way trichloroethylene was handled, the patients were assigned to three exposure classes, high (17 cases), medium (24) and low (3) intensity. For comparison, the presence of VHL mutations was also investigated in 107 renal cell carcinoma patients without a history of trichloroethylene exposure (but from the same geographical area as the trichloroethylene exposed patients), and in lymphocyte DNA from 97 healthy subjects. VHL mutations were identified by polymerase chain reaction analysis, single strand conformation polymorphism analysis, DNA sequencing and restriction enzyme digestion. VHL mutations were seen in tumour DNA of 75% of trichloroethylene exposed patients. Of those with VHL mutations, 39% had a C>T missense mutation at nucleotide 454. The presence of nucleotide 454 mutation was also investigated in the 107 renal cell cancer patients without trichloroethylene exposure, and none were found. Nucleotide 454 mutations were found also in non-tumour kidney cells of four of these trichloroethylene exposed patients. There was a statistically significant positive association between the number of mutations in each patient and the level of trichloroethylene exposure. The results of any DNA analyses conducted in relation to the 97 healthy subjects were not reported. These results suggest that there is a type of VHL gene mutation present in the renal cells of renal cell carcinoma patients that is not present in the tumours of renal cell carcinoma patients who have no occupational exposure to trichloroethylene. This association between trichloroethylene exposure and unique pattern of mutations in the VHL gene is difficult to interpret in the context of an assessment of the hazardous properties of trichloroethylene. Possibly the VHL mutations were an unspecific late stage development due to the interaction of trichloroethylene and cancer cells, and not part of the chain of events leading to cancer. Although the methods used in this study are considered to be

state-of-the-art, this investigation is considered pioneering in nature. Consequently, without replication in another group of patients, the weight that can be given to these results is limited.

A second team has studied VHL mutations in another group of renal cell cancer patients with a history of occupational exposure to trichloroethylene (Schraml et al., 1999). Twelve patients from Berlin with renal tumour, who were said to be "highly exposed to solvents", were identified; no further information on the patients, or the nature of their solvent exposure was reported. Control material relating to 615 "spontaneous" renal tumours was obtained from a pathology department in Basel; no further information on the control material was reported. Tumour and normal DNA was extracted from formalin fixed paraffin-embedded tissue and analysed using the genomic hybridization method and PCR sequencing analysis. In contrast to the findings of Brach et al., this investigation revealed no differences between solvent exposed and "spontaneous" tumour tissue in the phenotype, genotype or mutation pattern in the VHL gene. However, this study can contribute little to an assessment of the mutagenic properties of trichloroethylene because solvent exposure histories and other relevant information (such as age) on the study subjects are not available.

4.1.2.7.5 Summary of genotoxicity

The genotoxicity of trichloroethylene has been extensively investigated in experimental test systems. Trichloroethylene tested positive in a bacterial (Ames) test and a mouse lymphoma gene mutation assay, demonstrating that trichloroethylene is an *in vitro* mutagen. However, the weight of evidence indicates that this mutagenic activity is not expressed *in vivo*, because there are unequivocal negative results from well-conducted standard micronucleus, chromosome aberration, SCE and UDS assays. Certain DCVC metabolites of trichloroethylene have been shown to be mutagenic in bacterial assays.

In humans, several studies have been conducted which have looked for evidence of trichloroethylene-related genotoxicity in occupationally exposed groups. Most of these studies have major limitations with respect to their design and scope and consequently do not contribute any useful information to an assessment of the genotoxicity of trichloroethylene in humans. Two well-conducted studies provided evidence of the presence of mutations in the VHL tumour suppressor gene in the tumours of renal cell cancer patients with a history of trichloroethylene exposure; in contrast, another study suggested that there may be no VHL gene differences between renal cell cancer patients with and without a history of occupational exposure to trichloroethylene. The relevance of these VHL gene mutation investigations to an assessment of the hazardous properties of this substance is uncertain.

Overall, it is the conclusion of the rapporteur that trichloroethylene is an *in vitro* mutagen, but this activity is not expressed *in vivo* in somatic or germ cells.

However, there have been uncertainties about whether trichloroethylene should be classified for potential mutagenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity.

The Specialised Experts were concerned about recently published findings in several *in vivo* somatic cell tests using non-standard methodology, in support of genotoxic properties of trichloroethylene. The Specialised Experts concluded that the individual studies, both with negative and positive outcomes, exhibited shortcomings and weaknesses and each finding by itself was not a sufficiently reliable basis for a recommendation whether trichloroethylene should be classified for mutagenicity. However, the majority view was that in a weight of evidence

approach, the positive results support the assumption that trichloroethylene exerts genotoxic activity and can interact with DNA, inducing changes relevant to mutagenicity in somatic cells of mammals *in vivo*. In addition, several Specialised Experts pointed out individual findings, which for them were of particular concern, justifying classification. A majority of the Group recommended that classification as a category 3 mutagen was warranted. Specialised Experts not supporting this recommendation stressed negative test results as self-contradictory to a weight of evidence approach, weaknesses of individual *in vivo* studies, and the overall inconsistency of the results in relation to a clearly defined mechanism of DNA interaction. In addition, they pointed out the high doses of trichloroethylene required for the demonstration of a positive response. Furthermore, they felt that there was a lack of correlation between the different types of DNA interaction, the tissues in which they were observed, with respect to the tumour target organ (the kidney), and the hypothetical tumour forming mechanisms. Several Specialised Experts considered these views unjustified.

The Specialised Experts were in agreement that an evaluation of germ cell mutagenicity was not possible on account of the lack of sufficient information.

In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly. This conclusion has been carried forward to the risk characterisation.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Inhalation

In a series of studies, the carcinogenicity of trichloroethylene was investigated in the Wistar rat, NMRI mouse and Syrian hamster (Henschler et al., 1980, cited in HSE, 1982). Groups of thirty animals of each species and sex were exposed to trichloroethylene vapour (epoxide-free, stabilised by an amine base) at concentrations of 0, 100 or 500 ppm, 6 hours/day, 5 days/week for 18 months and observed for a total of 30 (mice and hamsters) or 35 (rats) months. In rats and hamsters, the overall incidence of tumours in the treated groups was similar to that of the controls, although no information was given on the time of appearance of tumours. In female mice exposed to either 100 or 500 ppm there was a statistically significant increase in the numbers of animals with malignant lymphomas (17/30 (57%) and 18/28 (64%), respectively). However, there was a relatively high incidence of this type of tumour among the control females (9/29, 30%) and it was stated by the authors that the NMRI mouse has a high spontaneous incidence of lymphomas (although no historical background data were presented in support), suggesting that these changes cannot confidently be attributed to trichloroethylene exposure. This series of studies had one major deficiency, namely the group sizes were inadequate; OECD guidelines recommend a minimum of fifty animals of each sex/group. Also, little information was provided on the general toxicity and it was not possible to judge if the exposure levels could have been higher. Overall, these studies provide some evidence that epoxide-free trichloroethylene is not carcinogenic in animals by the inhalation route, but because of the design and reporting deficiencies only limited weight can be given to this evidence.

In a series of comprehensive inhalation studies, Sprague-Dawley rats and mice (B6C3F1 and Swiss strains) were exposed to purified, epoxide-free trichloroethylene at levels of 0, 100, 300 or 600 ppm, 7 hours/day, 5 days/week (Maltoni et al., 1986). The mice were exposed for either 8 or 78 consecutive weeks and the rats for 8 or 104 weeks. All animals were observed until spontaneous death. The group size was generally at least 90 of each sex. Evidence of general toxicity was limited to the observation of kidney tubule meganucleocytosis in rats exposed to concentrations of 300 and 600 ppm for 104 weeks.

Among both rats and mice exposed for 8 weeks, there were no clear differences in the incidence of tumours between the exposed and control groups. For rats receiving long-term exposure, the overall incidence of tumours was not affected by treatment. There was, however, a dose-related and statistically significant increase in the incidence of Leydig cell tumours in rats in all trichloroethylene exposed groups; the numbers of affected animals were 6/135 (4%), 16/130 (12%), 30/135 (22%) and 31/130 (24%) in the control, 100, 300 and 600 ppm groups, respectively. Also, four males and one female from the highest exposure group had kidney tubular adenocarcinomas, a tumour type not previously seen at the testing laboratory in Sprague-Dawley rats. In Swiss mice there was no clear increase in the overall incidence of tumours in the trichloroethylene exposed groups. However, among males the incidence of lung tumours, mainly adenomas, was significantly greater than the controls (11%) at 300 (25%) and 600 ppm (30%) and, also in males, the incidence of hepatocellular adenomas and carcinomas at 600 ppm (14%) was significantly greater than the controls (4%). In B6C3F1 mice there was a significant increase in the overall incidence of malignant tumours in females at all exposure concentrations, although there were no noteworthy increases in any particular type of malignant tumour. A dose-related increase in the numbers of females with lung tumours, mainly adenomas, was observed, with the difference achieving statistical significance at the highest exposure level (17% at 600 ppm, compared with 4% among controls). Also, there was a slight increase in the incidence of B6C3F1 mice with hepatomas at the highest exposure level; this increase was statistically significant when males and females were analysed together (combined incidences were 2%, 3%, 4% and 8% in the control, 100, 300 and 600 ppm groups, respectively. To summarise the findings of this study, increased incidences of hepatomas in male Swiss mice and B6C3F1 mice of both sexes, lung tumours in male Swiss mice and female B6C3F1 mice, Leydig cell tumours in rats and, in association with other renal pathological changes, renal tubular adenocarcinomas in rats were observed in epoxide-free trichloroethylene treated groups.

Another inhalation study was conducted in female ICR mice and female Sprague-Dawley rats, using reagent grade trichloroethylene (99.82% pure, impurities included epichlorohydrin at 0.019%) (Fukuda et al., 1983). Groups of approximately fifty females were exposed at concentrations of 0, 50, 150 and 450 ppm, 7 hours/day, 5 days/week for 2 years, followed by an observation period of 3 weeks. No evidence of non-cancer toxicity was observed in the trichloroethylene exposed groups. In mice only, the incidence of lung adenocarcinomas at 150 ppm (8/50, 16%) and 450 ppm (7/46, 15%) was significantly greater than the controls (1/49, 2%). There were no inter-group differences in mice in the incidence of other types of tumours that were considered to be related to trichloroethylene treatment. There was no evidence of trichloroethylene-induced tumours in the rats. This study provides evidence that trichloroethylene, containing small quantities of epichlorohydrin, can induce lung tumours in mice, but not in rats.

Oral

The carcinogenicity of epoxide-stabilised trichloroethylene (purity 99%, with 0.2% 1,2-epoxybutane and 0.19% epichlorohydrin) by the oral (gavage) route was studied in the

Osborne-Mendel strain of rat and B6C3F1 mouse (NCI, 1976, cited in HSE, 1982). There were fifty animals of each sex per group. A high-dose group of rats received doses of around 1,250 mg/kg/day and high-dose mice received doses of around 2,000 mg/kg/day. Low-dose groups received doses of half that of the high-dose group. The animals were dosed for 78 weeks and the study was terminated at 90 weeks for mice and 110 weeks for rats.

In rats, no evidence of carcinogenicity was observed, but the value of the study was severely limited by the low survival rate in the treated groups; only 12/50 (24%) of males from the high-dose group and less than 50% of females from both treatment groups survived 78 weeks. In mice of both sexes there was an increased incidence of primary hepatocellular carcinomas in the trichloroethylene treated groups; the number of affected animals in the control, low and high-dose groups, respectively, was for males 1/20 (5%), 26/50 (52%) and 31/48 (64%) and for females 0/20, 4/50 (8%) and 11/47 (23%). This study has been widely criticised (see, for example, Henschler et al., 1977) because the trichloroethylene used for this study contained significant quantities of epoxide stabilisers. Nevertheless, the study clearly demonstrates that epoxide-stabilised trichloroethylene is carcinogenic in mice, causing hepatocellular carcinomas, an effect also seen in a subsequent carcinogenicity study involving epoxide-free trichloroethylene (see NTP, 1990, below).

Another oral (gavage) study was conducted in F344/N rats and B6C3F1 mice using epoxide-free trichloroethylene ("Hi-Tri" grade, >99.9% purity, containing 8 ppm amine stabiliser) (NTP, 1990). Groups of fifty animals of each sex were dosed at levels of 500 or 1,000 mg/kg/day for rats and 1,000 mg/kg/day for mice. Vehicle (corn oil) and untreated control groups were included. Among rats, male survival rates were significantly reduced in comparison with the vehicle control group (survival was 70%, 40% and 32% in the control, low- and high-dose groups, respectively), but female survival was not affected. There were adverse effects on bodyweight gain for high-dose males (body weight was 87% of vehicle control value at end of study), low-dose females (88%) and high-dose females (82%). Renal cytomegaly was observed in most trichloroethylene treated animals of both sexes. In males there was a low, but statistically significant, incidence of renal tubular cell adenomas and adenocarcinomas in the treated groups (0/48, 2/49 (4%) and 3/48 (6%)) in the vehicle control, low- and high-dose groups, respectively). No neoplasms considered to be related to trichloroethylene treatment were seen in female rats. It was concluded by NTP that because of the low survival in treated males (also, 20% of high-dose males accidentally killed due to dosing errors) and because the non-tumour pathology made the interpretation of the kidney tumour findings difficult, the data for the male rat were "inadequate to evaluate the presence or absence of a carcinogenic response". For the female rat, there was no evidence that trichloroethylene is carcinogenic.

In the trichloroethylene treated mice, male survival rate was reduced in comparison with the controls (probability of survival in the treated group was roughly half that of the control group). Also, male bodyweight was reduced in the treated group, to 90% of the vehicle control value. Renal cytomegaly was observed in most treated animals of both sexes, although the lesions were not as severe as those seen in the rats. Trichloroethylene caused a significant increase in the incidence of hepatocellular carcinomas in both males (8/48 (17%) and 31/50 (62%) in the vehicle control and treated group, respectively) and females (2/48 (4%) and 13/49 (27%), respectively). Also, the incidence of hepatocellular adenomas was significantly elevated in trichloroethylene treated males (7/48 (15%) and 14/50 (28%), respectively) and females (4/48 (8%) and 16/49 (33%), respectively). Thus, this study clearly shows that epoxide-free trichloroethylene is carcinogenic in mice, producing hepatocellular carcinomas and adenomas.

The carcinogenicity of epoxide-free trichloroethylene ("Hi-Tri" grade, >99.9% purity, containing 8 ppm amine stabiliser) was also investigated in four strains of rats, the ACI, August, Marshal

and Osborne-Mendel strains (NTP, 1988). Groups of fifty rats of each strain and sex received oral (gavage) doses of trichloroethylene at levels of 500 or 1,000 mg/kg/day, 5 days/week, for 103 weeks. Vehicle (corn oil) and untreated control groups were included. It was concluded by NTP that this study was inadequate for assessing the presence or absence of carcinogenicity because of low survival in many of the treated groups, the presence of severe kidney toxicity (tubular cell cytomegaly and nephrosis) and deficiencies in the study conduct. Despite this conclusion, it should be noted that there was a low incidence of kidney tubular cell carcinomas and adenomas in the treated groups, which is consistent with the findings of the NTP study in F344/N rats (NTP, 1990). There was also an increased incidence of testicular interstitial cell tumours in high-dose Marshal rats; the numbers of affected animals in the untreated control, vehicle control, low- and high-dose groups were, respectively, 16/46 (35%), 17/46 (37%), 21/48 (44%) and 32/48 (67%). However, in view of the relatively high incidence of this tumour type in the control groups, and because a similar increased incidence was not seen in the other strains, this observation was considered not to represent evidence of trichloroethylene-related carcinogenic activity.

The carcinogenicity of trichloroethylene (purified, epoxide-free) by the oral route has also been investigated in the Sprague-Dawley rat (Maltoni et al., 1986). Groups of thirty rats of each sex received gavage doses at levels of 0, 50 or 250 mg/kg/day, 4 or 5 days/week for 52 weeks and were observed until spontaneous death. Evidence of general toxicity was limited to the observation of kidney tubule meganucleocytosis at 250 mg/kg/day only. There was a dose-related, although not statistically significant, increased incidence of leukaemias in males; the incidence in the control, low and high-dose groups was, respectively, 3.3, 6.7 and 10%. However, this marginal increase, in the absence of supporting data from other studies, was not considered to represent clear evidence of carcinogenic activity. This study had two major limitations, the small group size and the restriction of the dosing period to 52 weeks, and consequently is considered not to be a rigorous investigation of the carcinogenic potential of trichloroethylene.

Henschler et al. (1984) investigated the carcinogenic potential of epoxide-free trichloroethylene and epoxide-stabilised trichloroethylene in a single study to test the hypothesis that the carcinogenic activity reported in the NCI (1976) study was caused by epoxide stabilisers. Groups of fifty male and fifty female Ha:ICR Swiss mice received either highly purified trichloroethylene (stabilised with 0.0015% triethanolamine), industrial-grade trichloroethylene (99.4% pure, containing 0.11% epichlorohydrin and 0.2% 1,2-epoxybutane), trichloroethylene with added epichlorohydrin (0.8%), trichloroethylene with added 1,2-epoxybutane (0.8%) or trichloroethylene with both epichlorohydrin (0.25%) and 1,2-epoxybutane (0.25%) added. The test substances were administered daily by gavage for 18 months, initially at dose levels of 2,400 mg/kg/day for males and 1,800 mg/kg/day for females. However, due to severe toxicity (mortality, retarded bodyweight gain, enlarged liver) dosing was interrupted for several weeks and all dose levels were reduced by half from week 40. A vehicle (corn oil) control group was included. The study was terminated 2 years after commencement of treatment. There were statistically significant increases in the numbers of mice with forestomach tumours in all the groups receiving trichloroethylene with epoxide stabilisers. No forestomach tumours were seen among the animals treated with epoxide-free trichloroethylene, indicating that the epoxide stabilisers were responsible for the stomach tumours occurring in the other groups. There were no treatment-related changes in the incidence of tumours at other sites.

Dermal

The carcinogenicity of trichloroethylene following dermal application has been investigated in one study, using Ha:ICR Swiss mice (Van Duuren et al., 1979). A group of thirty mice each received (possibly fifteen of each sex) a thrice-weekly dermal application of 1 mg (in acetone) purified trichloroethylene until spontaneous death. The dose was described as being less than the maximum tolerated dose. No skin tumours were observed. An "initiation-promotion" study was also conducted, in which mice were given a single dermal application of 1 mg trichloroethylene, followed by the thrice-weekly application of phorbol myristate acetate for life. Trichloroethylene showed no evidence of possessing initiating properties. Limitations of this study include the small group sizes and use of a single dose level that did not elicit toxicity.

Summary of animal carcinogenicity

The carcinogenicity of trichloroethylene has been investigated in a number of long-term animal studies, using the oral and inhalation routes, and involving hamsters and a variety of strains of rat and mouse. These studies provide clear evidence that trichloroethylene is carcinogenic in rats and mice.

In the mouse, trichloroethylene induced hepatocellular tumours by either the inhalation or oral routes. This effect was seen in Swiss and B6C3F1 strains, but not in NMRI or Ha:ICR strains. The hepatocellular tumours were observed at high oral dose levels, of 1,000 mg/kg/day and above (lower exposure levels were not investigated), and by inhalation at 600 ppm, but not at 300 ppm. Also, by the inhalation route only, an increased incidence of lung adenomas or adenocarcinomas was induced in three strains of mice, namely the ICR, Swiss and B6C3F1 strains. These lung tumours occurred at exposure levels as low as 150 ppm in one study but not at 100 ppm in another.

In the rat, trichloroethylene induced renal tubular adenomas or adenocarcinomas, albeit at a low incidence, in association with other pathological changes. This effect was observed consistently across a number of studies. The kidney tumours were induced at an exposure level of 600 ppm but not at 300 ppm inhalation exposure; by oral exposure kidney tumours were seen at 500 mg/kg/day and above in some studies but not at 250 mg/kg/day in another. In one inhalation study, there was an increased incidence of Leydig cell tumours, but this finding was not reproduced in other studies and has consequently been disregarded.

The carcinogenicity of trichloroethylene by the dermal route has not been adequately studied in animals.

Proposed mechanisms of trichloroethylene toxicity/carcinogenicity

Since it is not clear what role genotoxicity may play in the mechanism underlying trichloroethylene induced tumours, other mechanisms of tumour formation seen in animal studies should be considered. It is evident from the toxicity profiles for trichloroethylene that there are a number of species and strain differences in responses to this substance.

Mouse liver

Hepatocellular tumours have been induced in Swiss and B6C3F1 mice given trichloroethylene by the inhalation and oral routes, but not in NMRI or Ha:ICR mice or any strain of rat tested so far. Syrian hamsters also did not develop liver tumours following exposure to trichloroethylene in the one study in which this species was tested. There is strong evidence linking the

trichloroethylene metabolite trichloroacetic acid and possibly also dichloroacetic acid with the tumorigenic activity of trichloroethylene in certain strains of mice. Both metabolites are carcinogenic in the mouse but not the rat. Trichloroacetic acid has been shown to act as a complete carcinogen in groups of 11, 24 or 22 male B6C3F1 mice given around 150, 300 or 1,000 mg/kg/day over 52 or 61 weeks (Bull et al., 1990; Herren-Freund et al., 1987). Bull et al. (1990) also conducted a limited study in a group of 10 female B6C3F1 mice given around 300 mg/kg/day over 52 weeks. No tumours were found in females, although the non-neoplastic pathology of the liver (accumulation of lipofuscin) was reported to be similar for both sexes. No tumours and no evidence of lipofuscin accumulation were found in a small study in a group of 3 male and 2 female Sprague-Dawley rats given around 300 mg/kg/day trichloroacetic acid in drinking water for 52 weeks (Bull et al., 1990). In a separate study, liver toxicity (nature not specified) but no tumours were observed in male F344 rats (group size not stated) given up to 378 mg/kg/day trichloroacetic acid via drinking water for 100-104 weeks (DeAngelo and Daniel, 1992).

Dichloroacetic acid has also been shown to act as a complete carcinogen in B6C3F1 mice but not Sprague-Dawley or F344 rats (Bull et al., 1990; DeAngelo and Daniel, 1992). In groups of 11 or 24 male mice given dichloroacetic acid for 52 weeks, a clear increase in liver tumours was seen at around 300 mg/kg/day and a slight increase at 150 mg/kg/day. A limited study was also conducted in a group of 10 female mice given 300 mg/kg/day and although no tumours were found, an increase in the incidence of microscopic hyperplastic nodules was observed, suggesting that had the study continued for longer these animals may have developed tumours. Liver tumours were also observed in a group of 33 male B6C3F1 mice treated with around 90 mg/kg/day dichloroacetic acid for 104 weeks (Daniel et al., 1992). No tumours were seen in 3 male and 2 female Sprague-Dawley rats given around 300 mg/kg/day dichloroacetic acid in drinking water for 52 weeks (Bull et al., 1990). Furthermore, non-neoplastic changes in these rats were very much less than those seen in mice at an equivalent dose. A more extensive study using male F344 rats (numbers not reported) found no evidence of tumours at doses up to 295 mg/kg/day for 60 weeks or 48 mg/kg/day for around 104 weeks. The top dose group had to be terminated early in this study due to excessive treatment-related mortality.

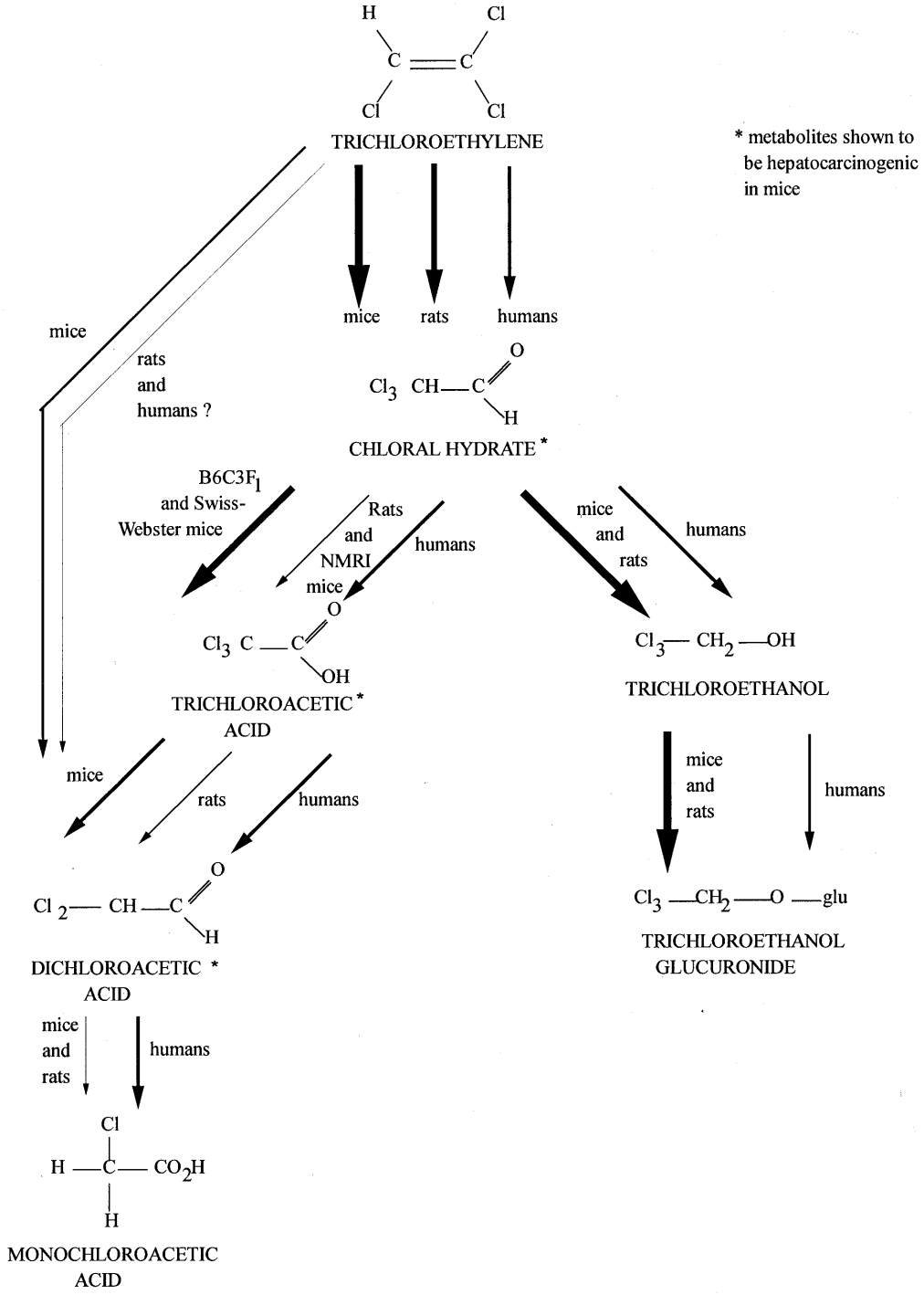
Recent studies have compared blood levels of trichloroacetic acid and dichloroacetic acid following administration of trichloroacetic acid, dichloroacetic acid or trichloroethylene (Larson and Bull, 1992a; 1992b; Bull et al., 1993). In male B6C3F1 mice, peak blood levels and areas under the curve for trichloroacetic acid were similar following oral administration of 20 mg/kg trichloroacetic acid or 200 mg/kg trichloroethylene. Following administration of a higher dose of trichloroethylene (2,000 mg/kg), peak blood levels of trichloroacetic acid were similar to those found after a dose of 100 mg/kg trichloroacetic acid but the area under the trichloroacetic acid blood concentration versus time curve was 2-fold greater after trichloroethylene. This suggests that in mice, blood levels of the carcinogenic metabolites trichloroacetic acid and dichloroacetic acid can reach potentially tumorigenic levels from dose levels of trichloroethylene that have been shown to cause tumours. Similarly, peak blood levels of dichloroacetic acid seen after an oral dose of 2,000 mg/kg trichloroethylene were almost twice those seen after an oral dose of 100 mg/kg dichloroacetic acid.

In contrast to these findings, in male Sprague-Dawley rats, peak levels of trichloroacetic acid were nearly 3-fold higher and the area under the curve nearly 1.5-fold higher following a 20 mg/kg dose of trichloroacetic acid than with a 2,000 mg/kg dose of trichloroethylene. With higher doses of trichloroacetic acid (100 mg/kg) and trichloroethylene (3,000 mg/kg) peak blood levels were still 3 times higher following the dose of trichloroacetic acid, although the areas under the curve were the same. A much more striking difference was noted for dichloroacetic

acid. While blood levels of dichloroacetic acid were below the limits of detection (0.5 μg/ml) following administration of 3,000 mg/kg trichloroethylene, when rats were given a dose of dichloroacetic acid, blood levels were nearly twenty times those seen in mice following an equivalent dose of dichloroacetic acid. It therefore seems that not only do mice generate greater amounts of these metabolites compared to rats following exposure to trichloroethylene, the blood levels of trichloroacetic acid and dichloroacetic acid found in rats after exposure to trichloroacetic acid and dichloroacetic acid respectively suggest that species differences also exist in sensitivity to the carcinogenic properties of these metabolites (see **F igure 2** on next page).

There have been a number of studies conducted to characterise the role of both trichloroacetic acid and dichloroacetic acid in the hepatocarcinogenesis of trichloroethylene. Elcombe et al. (1985) showed that trichloroethylene will induce peroxisome proliferation, assessed by ultrastructural examination, in male B6C3F1 and Alderley Park (Swiss) mice but not male Osborne-Mendel or Alderley Park (Wistar derived) rats given trichloroethylene in corn oil at levels of 500-1,500 mg/kg/day for 10 days. Biochemical changes indicative of peroxisome proliferation have also been seen in B6C3F1 mice but not F344 rats given between 500 and 2,000 mg/kg/day trichloroethylene for 10 days (Goldsworthy and Popp, 1987; Knuckles, 1990). In contrast, oral administration of the trichloroethylene metabolite trichloroacetic acid to male Alderley Park and B6C3F1 mice and male Alderley Park and F344 rats for 10 days produced similar levels of peroxisome proliferation assessed by biochemical markers in both species (Elcombe, 1985; Goldsworthy and Popp, 1987; Knuckles, 1990; Watson et al., 1993). Dose levels used in these studies ranged from 10 to around 900 mg/kg/day.

Figure 2. Species differences in the metabolism of trichloroethylene to trichloroethanol, trichloroacetic acid, dichloroacetic acid and monochloroacetic acid.



Peroxisome proliferation assessed by biochemical markers has apparently been seen in Sprague-Dawley rats given around 350 mg/kg/day trichloroacetic acid for 90 days (Mather et al., 1990, cited in ACGIH, 1991). The ability of trichloroacetic acid to induce peroxisome proliferation has also been assessed in male Osborne-Mendel and F344 rats given around 330 or 720 mg/kg/day trichloroacetic acid and male Swiss-Webster, B6C3F1, C57BL/6 and C3H mice given around 260 or 440 mg/kg/day trichloroacetic acid in drinking water for 14 days (DeAngelo et al., 1989). Results in this last study showed relatively small increases in palmitoyl-Co A oxidase activity in

rat strains (238 and 163% increases for Osborne-Mendel and F344 rats respectively when compared to controls). In contrast marked increases in palmitoyl-Co A oxidase activity were seen in all strains of mice; increases of around 750% were seen in comparison to controls for Swiss-Webster, B6C3F1 and C3H mice, and 2000% in comparison to controls for the C57BL/6 strain.

It has been shown that in rats, saturation of metabolism of trichloroethylene to trichloroacetic acid occurs at much lower dose levels than is the case in mice (Prout et al., 1985). This difference in metabolism of trichloroethylene to a metabolite capable of inducing peroxisome proliferation probably accounts for the observed species difference in peroxisome proliferation in response to trichloroethylene. However, it does not explain why rats will undergo peroxisome proliferation in response to trichloroacetic acid but do not subsequently develop liver tumours.

Other effects that may play a role in the development of cancer and that have been investigated in rats and mice include hepatic DNA synthesis and mitosis. Stott et al. (1982) found increases in DNA synthesis (measured by increases in tritiated thymidine incorporation) in B6C3F1 mice given 2,400 mg/kg trichloroethylene orally (222% of control values after 3 days of exposure and 181% of control values following 3 weeks of exposure at this dose level). Increases in hepatic DNA synthesis were also seen in Osborne-Mendel rats given 1,100 mg/kg orally. After three days of dosing, DNA synthesis had increased to 121% of control levels although this increased to 175% of control levels after 3 weeks of dosing.

In contrast, Elcombe et al. (1985) observed much greater differences between rats and mice in the extent to which DNA synthesis was increased following exposure to trichloroethylene. They gave male Alderley Park and B6C3F1 mice and male Alderley Park and Osborne-Mendel rats 500-1,500 mg/kg trichloroethylene in corn oil for 10 days. Controls received corn oil alone. Although hepatic hypertrophy was observed in both rats and mice, marked dose-related increases in DNA synthesis were seen in mice only. At the top dose level, in B6C3F1 mice, DNA synthesis was increased 478% over control levels and in Alderley Park mice, DNA synthesis was increased by 574% over control levels. In rats, the greatest increase in DNA synthesis was seen in the Alderley Park strain to 206% of control levels. The maximum increase seen in Osborne-Mendel rats was 118% of control levels and there was no dose-response relationship for either strain of rat. Furthermore, species differences in cell division in response to trichloroethylene were also reported. In both strains of mouse, the incidence of mitotic figures was clearly increased in treated mice in comparison to the low levels seen in controls, whereas in rats, trichloroethylene reduced the incidence of mitotic figures in treated animals when compared to the levels found in controls. Control rats had a 10-fold higher incidence of mitotic figures than control mice. Although the incidences of mitotic figures in treated animals were different to those in control groups, there was no dose-relationship within treated groups for either species.

Dees and Travis (1993) also report increases in DNA synthesis in B6C3F1 mice of both sexes given 10 daily doses of 0, 100, 250, 500 or 1,000 mg/kg/day trichloroethylene in corn oil. There was a tendency for DNA synthesis to increase with increasing dose up to 250 mg/kg/day and then plateau at a level approximately twice that of control levels, although standard deviations were quite large. Mirsalis et al. (1985) found increases in hepatocyte cell proliferation in B6C3F1 mice of both sexes but no induction of unscheduled DNA synthesis, following gavage of 0, 50, 200 or 1,000 mg/kg trichloroethylene in corn oil. Neither of the above two studies included rats.

The above studies by Stott et al. (1982), Elcombe et al. (1985), Dees and Travis and Mirsalis et al. (1985) suggest that there may be some species differences in DNA synthesis and cell division between rats and mice, although there is some conflict between the results of Stott et al. (1982)

and Elcombe et al. (1985) in the extent to which DNA synthesis is increased in Osborne-Mendel rats given trichloroethylene.

There are also some indications of species differences in the extent to which mice and rats undergo DNA synthesis in response to trichloroacetic acid. Watson et al. (1993) compared hepatic DNA synthesis in male B6C3F1 mice given 0 or around 900 mg/kg/day and male F344 rats given 0 or around 600 mg/kg/day trichloroacetic acid in drinking water for 7 days. Results showed marked species differences in the incorporation of BUdR into hepatocyte nuclei. Whereas in mice, a four-fold increase in cell proliferation occurred, in rats, cell proliferation dropped to 10% of the control level. It is not clear why mice and rats should show such different responses. When male B6C3F1 mice were given lower levels of trichloroacetic acid, between 50 and 370 mg/kg/day, in drinking water for up to 15 days, no increase in cell replication was found although DNA synthesis was elevated after 5 and 14 days treatment (Sanchez and Bull, 1990).

Studies in which intercellular communication between hepatocytes *in vitro* was investigated have shown that trichloroethylene (with metabolic activation) and trichloroacetic acid (without activation) were able to inhibit gap junction mediated communication in mouse but not rat hepatocytes (Klaunig et al., 1989). Chloral hydrate and trichloroethanol had no effect in hepatocytes from either species. The extent to which this finding may contribute to the development of liver tumours in the mouse and the significance of this finding for human health is unclear.

It has been postulated that differences in the extent to which rats and mice metabolise trichloroethylene explain in part the species differences in toxicity: mice may generate sufficient trichloroacetic acid to exceed a threshold for peroxisome proliferation whereas rats do not. The peroxisome proliferation is accompanied by sustained cell proliferation in mice, which can be expected to contribute to the eventual development of neoplasms.

Although the role of the minor metabolite dichloroacetic acid has not been investigated as extensively as that of trichloroacetic acid, there is evidence to suggest that the two metabolites may have different mechanisms of action. Dichloroacetic acid has also been shown to induce peroxisome proliferation in B6C3F1 mice and to a much lesser degree in Sprague-Dawley rats but only at dose levels much higher than those shown to induce liver cancers in mice (DeAngelo et al., 1989*;* Daniel et al., 1992). However, unlike trichloroacetic acid, administration of dichloroacetic acid for 52 weeks to male B6C3F1 mice at a level of 1 or 2 g/l in drinking water produced severe cytomegaly accompanied by glycogen accumulation throughout the liver (Bull et al., 1990). In addition, areas of recurrent necrosis and regeneration were apparent. In a limited study in Sprague-Dawley rats given 5 g/l dichloroacetic acid for 12 months, hepatocyte enlargement was much less marked and only localised accumulation of glycogen was apparent. Furthermore it has been shown that dichloroacetic acid will induce both cell division and DNA synthesis in male B6C3F1 mice (Sanchez and Bull, 1990).

Although it has been shown that mice given tumorigenic doses of trichloroethylene produce blood levels of dichloroacetic acid equivalent to those seen using tumorigenic doses of dichloroacetic acid, the information on the extent to which trichloroethylene is metabolised to dichloroacetic acid in rats is conflicting. Larson and Bull (1992a) found that mice metabolise trichloroethylene to dichloroacetic acid to a much greater extent than do rats. In contrast, Dekant et al. (1984) and Green and Prout (1985) reported that mice and rats produce similar amounts of dichloroacetic acid. However, the limited data that are available suggest that mice are more sensitive to the effects of dichloroacetic acid than rats. Therefore dichloroacetic acid may also play a role in the development of trichloroethylene induced liver tumours in mice.

Of the other metabolites of trichloroethylene, no information was available on the carcinogenicity of trichloroethanol. Monochloroacetic acid has been shown not to cause peroxisome proliferation in male B6C3F1 mice and male Sprague-Dawley rats given up to 482 and 501 mg/kg/day respectively in drinking water for 14 days (DeAngelo et al., 1989). Monochloroacetic acid has also been shown not to induce liver tumours in male F344 rats given 69 mg/kg/day in drinking water for 100-104 weeks (DeAngelo and Daniel, 1992). Chloral hydrate has been shown to be carcinogenic in male B6C3F1 mice given chloral hydrate for 104 weeks (Daniel et al., 1992). This metabolite is also reported to have produced tumours in male B6C3F1 mice following a single dose (Rijhsinghani et al., 1986, cited in Bull et al., 1990). No further details were provided from this study and no data on the carcinogenic potential of this metabolite in rats were available. Given the sparsity of data for other trichloroethylene metabolites it is not possible to draw any conclusions about their relative importance for tumour induction following exposure to trichloroethylene.

The significance of these findings for humans has been investigated. There is evidence to show that human hepatocytes do not undergo peroxisome proliferation in response to trichloroacetic acid. Elcombe et al. (1985) compared the peroxisome-specific β-oxidation activity of male Alderley Park rat, male Alderley Park mouse and human hepatocytes (obtained from 2 individuals) exposed to various concentrations of trichloroacetic acid *in vitro* for 3 days. Whereas clear increases in activity were seen in hepatocytes from both rodent species, no increase was seen in human hepatocytes. Furthermore, these researchers demonstrated that *in vitro*, human hepatocytes metabolise trichloroethylene to trichloroacetic acid at a rate 3-fold slower that that seen in rat hepatocytes and 120-fold slower than in mouse hepatocytes. Results from studies by Knadle et al. (1990, cited in ECETOC, 1994) apparently support these conclusions.

Less is known about the extent to which humans metabolise trichloroethylene to dichloroacetic acid, other than that it is likely to be a relatively minor pathway, or about the effects of this metabolite in human liver. It is therefore not possible to draw any conclusions about the likely effects of metabolism of trichloroethylene to dichloroacetic acid in humans.

Summary of tumours in mouse liver

There is a growing body of evidence to show that development of liver tumours in mice exposed to trichloroethylene is linked to the way mice metabolise the substance. In this species trichloroethylene is much more readily metabolised to trichloroacetic acid, a metabolite which has been shown to induce peroxisome proliferation and to cause increases in cell proliferation. It is thought that these effects in combination lead to the development of liver tumours in mice. Evidence from *in vitro* studies has shown that the extent to which trichloroethylene is metabolised to trichloroacetic acid in human hepatocytes is closer to that found in rat hepatocytes (i.e. much less than in mouse cells) and rat is a species which does not develop liver tumours following exposure to trichloroethylene. In addition, human hepatocytes have been shown not to undergo peroxisome proliferation in response to trichloroacetic acid whereas both mouse and rat hepatocytes do. It therefore seems reasonable to conclude that the effects of trichloroacetic acid in mice are unlikely to be of relevance for humans. The role of the minor metabolite dichloroacetic acid has been less extensively investigated but from the evidence currently available it seems that the effects of this metabolite may also be mouse specific. Overall, therefore, although trichloroethylene is found to induce liver tumours in mice, the weight of evidence available indicates that these effects are unlikely to be of significance for human health.

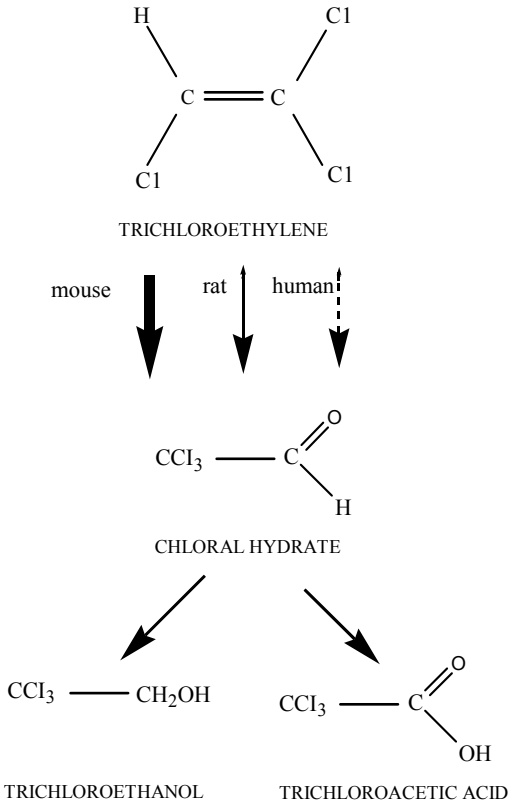
Mouse lung

The tumours seen in mouse lung following exposure to trichloroethylene may also have a metabolic basis. When male B6C3F1 mice, CD-1 mice of both sexes and female Alderley Park rats were given single or repeated doses of trichloroethylene by inhalation or by intraperitoneal injection, pulmonary toxicity was observed in mice but not rats (Odum et al., 1992; Green et al., 1997; Villaschi et al., 1991; Forkert et al., 1985; Forkert and Birch 1989). Furthermore, toxicity was only seen in one specific cell type, the Clara cell, a cell type known to be active with respect to metabolism of xenobiotics. As yet it is not clear why other cell types in the lung which possess xenobiotic metabolic activity remain unaffected. There is also some evidence for strain specificity for lung tumours, female Ha:ICR mice, male Swiss mice and B6C3F1 mice of both sexes seem to be sensitive whereas no lung tumours were seen in female Swiss mice or NMRI mice of either sex.

Recently the metabolism of trichloroethylene has been investigated in Clara cells taken from female CD-1 mice *in vitro* (see **Figure 3** on next page) (Odum et al., 1992). It was shown that the major metabolite in these Clara cells was chloral hydrate and that the cells, once having formed this metabolite, were relatively inefficient at metabolising it further. Furthermore, a single inhalation exposure of female CD-1 mice to 100 ppm chloral hydrate for 6 hours also produces lung toxicity similar to that of trichloroethylene. In contrast, no lung toxicity was seen following a single exposure to 100 ppm trichloroethanol for 6 hours, 500 ppm trichloroethanol for 2 hours or a single intraperitoneal dose of 200 or 500 mg/kg trichloroacetic acid in 1% Tween-80. It is thought that accumulation of chloral hydrate within the Clara cell leads to cytotoxicity although the precise mechanism has not yet been elucidated. Where cells have been damaged there is evidence of regeneration and replication to repair and replace the damaged Clara cells (Villaschi et al., 1991) and it is possible that repeated cycles of damage and regeneration may lead eventually to lung tumour formation. There is some evidence that chloral hydrate may be mutagenic and clastogenic and it has been suggested that the initial step in the formation of mouse lung tumours may be a genotoxic event. However, the recent negative findings from *in vivo* micronucleus and bone marrow cytogenetics studies in the rat using highly purified (purity 99.4%) chloral hydrate (Leuschner and Leuschner, 1991) suggest that there may be other factors involved in the development of lung tumours in mice. There is also some evidence that chloral hydrate has carcinogenic activity. In one report an increase in liver tumours but not lung tumours was seen in male B6C3F1 mice given 0 or 166 mg/kg/day chloral hydrate in drinking water for 60 or 104 weeks (Daniel et al., 1992).

Liver tumours have also apparently been generated in male B6C3F1 mice following a single exposure to chloral hydrate but no further details of this study were available (Rijhsinghani et al., 1986, cited in Bull et al., 1990). The lack of lung tumour formation following oral exposure to chloral hydrate and trichloroethylene can be explained if metabolism of trichloroethylene to chloral hydrate by Clara cells is a necessary step in the development of tumours in the mouse lung. When mice are exposed to trichloroethylene by the inhalation route, the Clara cells are directly exposed to trichloroethylene and consequently able to metabolically activate trichloroethylene to chloral hydrate. However, when mice are given trichloroethylene or chloral hydrate orally, these substances undergo metabolism before they reach the lungs and hence the Clara cell is not exposed to a xenobiotic that it cannot effectively detoxify.

Figure 3. Metabolism of trichloroethylene in the lungs of mice, rats and humans



In addition to the studies in mice, the metabolism of trichloroethylene has been investigated in isolated rat and guinea pig lungs perfused with whole blood (Dalbey and Bingham, 1978). Lungs were exposed to trichloroethylene in the ventilation gas supplied to the lungs. In both species trichloroethanol and trichloroacetic acid were detected in the perfusate but not chloral hydrate or trichloroethanol-glucuronide. Levels of trichloroethanol in the perfusate increased with time and guinea pig lungs consistently produced more trichloroethanol than did rat lungs. Addition of ethanol to the blood used to perfuse the lungs did not affect that rate or extent of trichloroethanol formation. These data suggest that rat and guinea pig lungs may be able to metabolise trichloroethylene further than mouse lung, which may in part explain why rats did not develop lung tumours in long-term studies.

Further information on species differences in the pulmonary metabolism of trichloroethylene to chloral hydrate was provides by a series of *in vitro* studies in mouse, rat and human tissue (Green et al., 1997). Metabolism to chloral hydrate was 23-fold greater in mouse lung microsome preparations that in rat preparations, and no conversion was detected in human lung microsomes. Conversion of chloral to trichloroethanol was similar for rat, mouse and human. Immunolocalisation of cytochrome P450IIE1, an isoenzyme involved in the oxidative metabolism of trichloroethylene, showed high concentrations in the Clara cells of the mouse, lower levels in the rat, but none was detected human lung sections. The results of this study suggest that there are major species differences in the pulmonary metabolism of trichloroethylene to chloral hydrate, with humans having an extremely low capability. However, no species differences in the capacity to metabolise chloral to trichloroethanol were apparent.

Overall, there is evidence that the lung tumours seen in mice are related to the conversion of trichloroethylene to chloral hydrate in the Clara cells resulting in cytotoxicity and repeated

cycles of cell destruction and replication leading to tumour formation. Lung tumours were not seen in rats, which have a lower capacity than mice to metabolise trichloroethylene to chloral hydrate. Human lung tissue appears to have minimal capacity to metabolise trichloroethylene to chloral hydrate suggesting that the lung tumours seen in mice are of no relevance to humans.

Rat kidney

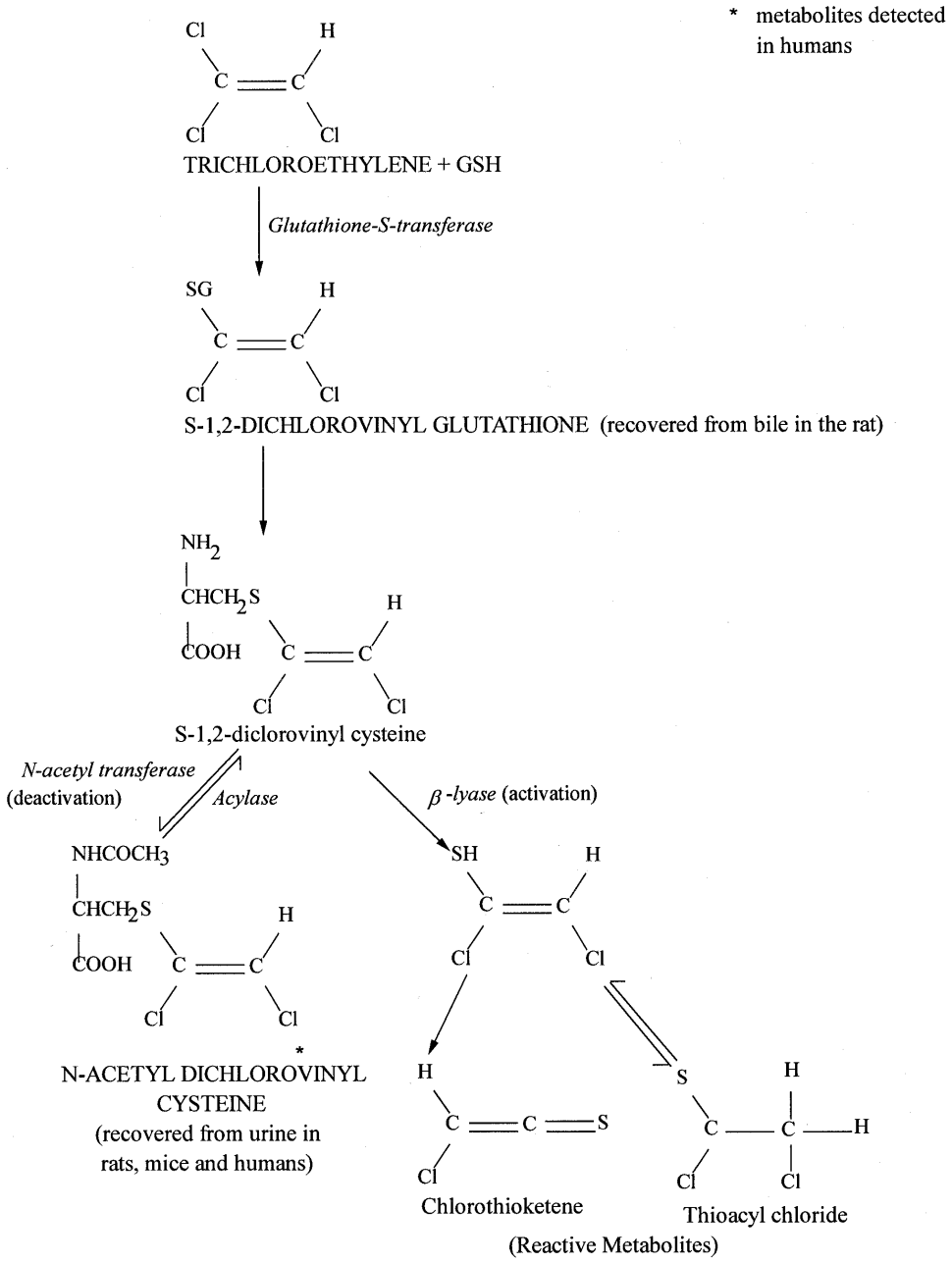
There is evidence that trichloroethylene produces species-specific toxicity in the kidneys of rats although there is still some uncertainty surrounding the mechanism by which rat kidney lesions arise. In long-term studies, low incidences of renal adenoma and adenocarcinoma have been seen in a number of strains of rat but not in any strain of mouse following oral and inhalation dosing (NTP, 1990; 1988; Maltoni et al., 1988). There are also indications that rats given trichloroethylene by both the oral and inhalation routes develop non-neoplastic lesions in the kidney at relatively low-dose levels.

One male rat specific mechanism that has been investigated is that of hyaline droplet accumulation. The role of α2μ-globulin and that of increased kidney cell replication has been examined in F344 rats of both sexes given 0 or 1,000 mg/kg trichloroethylene in corn oil for 10 days (Goldsworthy et al., 1987; 1988b). The results showed that hyaline droplet accumulation and kidney cell replication in rats of both sexes treated with trichloroethylene were no different from control values. This work is supported by results from a subsequent study in which male F344 rats were given 2,000 mg/kg trichloroethylene in corn oil for 42 days (Green et al., 1990). These studies suggest that hyaline droplet accumulation is unlikely to be of relevance in the development of kidney lesions in male rats exposed to trichloroethylene.

It has been suggested that the kidney tumours in rats arise as a result of persistent cytotoxicity and regeneration. A possible mechanism by which cytotoxicity could occur involves the formation of dichlorovinyl cysteine (DCVC) via a reductive glutathione conjugation pathway (see **Figure 4** on next page). It has been shown that microsomes obtained from the livers of Wistar rats will, in the presence of glutathione, transform trichloroethylene to S(1-2,dichlorovinyl)glutathione and very low levels of this metabolite (0.3 μg/ml) have been detected by mass spectrometry in bile taken from male Wistar rats given a single gavage dose of 2,200 mg/kg trichloroethylene in corn oil (Dekant et al., 1990). However, Ellis et al. (1995) using radiolabelled trichloroethylene or radiolabelled glutathione failed to detect glutathione conjugation in liver fractions from male F344 rats or humans. The limits of detection in their studies were 1 or 0.5 pmol/min/mg protein respectively. It would be expected that if glutathione conjugation of trichloroethylene was occurring, this should also result in the excretion of the mercapturic acid N-acetyl DCVC in the urine. This metabolite has been isolated from the urine of rats, mice and humans.

In male Wistar rats around 0.8 µg in total of 2 stereoisomers of N-acetyl DCVC were isolated from urine collected over 24-hours following gavage of 2,200 mg/kg trichloroethylene in corn oil (Dekant et al., 1990). Birner et al. (1993) isolated between 1-4 µg/ml urine from male and female Wistar rats and NMRI mice given 50 mg/kg trichloroethylene orally and between 0.7 and 1 µg/ml urine from a group of 4 trichloroethylene workers, although exposure levels for the trichloroethylene workers were not reported. Birner et al. (1993) also compared urinary N-acetyl DCVC levels with urinary trichloroacetic acid levels in humans, rats and mice and found that human urine contained a much greater proportion of N-acetyl DCVC than urine from either rodent species suggesting that humans may metabolise a greater proportion of trichloroethylene by this pathway. In male F344 rats given 500 or 2,000 mg/kg trichloroethylene in corn oil for

Figure 4. Metabolism of trichloroethylene via the glutathione conjugation pathway



1 or 10 days this metabolite was detected at very low levels, around 2-30 μg per 24 hour urine sample, accounting for only 0.001 to 0.008% of the administered dose (Green et al., 1990). N-acetyl dichlorovinyl cysteine has also been isolated from urine collected from male Sprague-Dawley rats given a single gavage dose of 400 mg/kg 1,2-14C-trichloroethylene in which it accounted for less than 0.1% of the total administered radioactivity (Dekant et al., 1986a). It therefore seems likely that a low level of glutathione conjugation occurs *in vivo* and consequently it would be expected that a low level of the metabolite DCVC is being generated.

The argument has been put forward that in rats, the metabolite dichlorovinyl cysteine is activated locally in the kidneys by the enzyme β-lyase. This hypothesis is supported by the fact that in the rat trichloroethylene is specifically nephrotoxic to kidney tubules and it is known that the enzyme β-lyase is located in the epithelium of renal tubules. Recently, the rate of activation of DCVC by the β-lyase pathway and the rate of deactivation by the N-acetyl transferase pathway have been compared *in vitro* in kidney cytosolic fractions from male F344 rats, male B6C3F1 mice and commercially available male human kidney cytosol (Ellis et al., 1995). Results showed that rats had the greatest capacity for metabolic activation via the β-lyase pathway (10-fold greater than that in mice or humans) but rats also had the greatest capacity for metabolic deactivation by the N-acetyl transferase pathway (1.4-fold greater than mice and 60-fold greater than humans). Furthermore in rats, mice and humans metabolic clearance via the N-acetyl transferase pathway was substantially greater than clearance by the β-lyase pathway (2 orders of magnitude greater in both rodent species and 27-fold greater in humans). Therefore there is some evidence that male rats have a greater capacity to activate DCVC than mice. However, since glutathione conjugation of trichloroethylene is a minor metabolic pathway and rats have a much greater capacity for metabolic deactivation than activation of DCVC, the amounts of active metabolites that are generated *in vivo* are likely to be extremely low.

There is also evidence that the urinary metabolite N-acetyl-DCVC can be deacetylated to regenerate DCVC and this has been studied *in vitro* using kidney cytosolic fractions from F344 and Wistar rats, NMRI mice and humans (Birner et al., 1993). The sex of the rats and mice from which kidney cytosol was obtained was not reported. Results showed that the greatest rate of deacetylation occurred with kidney cytosol from mice followed by that of F344 rats. Human kidney cytosol and that from Wistar rats showed equivalent activity of just under half that seen in the mouse and two thirds that of the F344 rat. Therefore even if the urinary metabolite N-acetyl DCVC were being reabsorbed from the kidney tubules, and so far there is no evidence that this does occur, there do not seem to be any marked species differences that could satisfactorily explain the apparent species differences in kidney toxicity.

The dose-dependency of the urinary excretion of markers of the glutathione metabolic pathway (two isomers of acetyl-DCVC) and marker of the oxidative metabolic pathway (trichloroethanol + trichloroacetic acid) was investigated in rats (Bernauer et al., 1996). Four rats were exposed to trichloroethylene by the inhalation route to concentrations of 40, 80 and 160 ppm for 6 hours. A dose-related increase in the excretion of acetyl-DCVC, trichloroethanol and trichloroacetic acid was seen. The amount of oxidative metabolites excreted was three orders magnitude higher than the glutathione metabolites. The dose-dependency of the urinary excretion of markers of the glutathione metabolic pathway and the oxidative metabolic pathway was also investigated in humans. Three volunteers were exposed to trichloroethylene by the inhalation route to concentrations of 40, 80 and 160 ppm for 6 hours. A dose related increase in the excretion of acetyl-DCVC, trichloroethanol and trichloroacetic acid was seen. The amount of oxidative metabolites excreted was three orders of magnitude higher than the glutathione metabolites. This study confirms the operation of the reductive metabolic pathway in both rats and human, and that

in both species the reductive pathway is quantitatively minor compared with the oxidative pathway.

Green et al. (1997) conducted a series of studies which were designed to provide further information on the possible role of glutathione conjugation in the development of trichloroethylene-induced kidney toxicity in the rat. In a metabolism study Fischer 344 rats received daily gavage doses of 500 or 2,000 mg/kg/day trichloroethylene. Urine was analysed for N-acetyl DCVC and trichloroacetic acid levels on days 1, 5 and 10. The amount of N-acetyl DCVC present was at least 3 orders of magnitude lower than that of trichloroacetic acid, and accounted for between 0.001 and 0.008% of the administered trichloroethylene. The relative sensitivity of rats and mice to DCVC liver and kidney toxicity was determined following either single oral administration (1 or 50 mg/kg) or repeated oral administration (0.1-5 mg/kg/day for 10 days) of DCVC. The mouse was found to be 5-10 fold more sensitive than the rat to DCVC kidney toxicity. *In vitro* studies showed that the rate of conjugation of trichloroethylene with glutathione is higher in the mouse (2.5 pmol/min per mg protein) liver than in the rat liver (1.6 pmol/min per mg protein) and human liver (0.02-0.37 pmol/min per mg protein). *In vitro* comparisons of the metabolism of DCVC by renal β-lyase and N-acetyl transferase indicated that metabolism by N-acetyl transferase was two orders of magnitude greater than by β-lyase in rats and mice, and 27-fold greater in humans; additionally, it was shown that metabolic clearance via β-lyase activity in the rat was 10-fold greater in the rat kidney than in the human and mouse kidney. Again, these results do not satisfactorily explain the species differences in susceptibility to kidney toxicity. If trichloroethylene kidney toxicity is related to the formation of DCVC and activation by β-lyase, then to be consistent with the findings of this study the mouse rather than the rat should be the more sensitive species to kidney toxicity and carcinogenicity.

Lash et al. (1999) investigated the metabolism of trichloroethylene by the reductive (glutathione) pathway in humans. Eleven male and 10 female volunteers were exposed to 50 ppm or 100 ppm trichloroethylene by inhalation for 4 hours. Blood and urine was sampled at various times before, during and after exposure for analysis of GHS, related thiols and disulphides, and GHS-derived (reductive) metabolites of trichloroethylene. In a parallel study, the oxidative metabolites of trichloroethylene were investigated in the same volunteers (Fisher et al., 1998). S-(1,2-dichlorovinyl)glutathione (DCVG) was found in the blood of all subjects from 30 min after the start of exposure. Peak blood concentrations and area under curve (AUC) for DCVG were greater in males. The distribution of values for both sexes was bimodal, suggestive of a polymorphism. DCVC was not detected in any volunteers. DCVC mercapturates were found in the urine of just one male volunteer. AUCs for the oxidative metabolites were markedly higher than for DCVG, although peak levels were similar (trichloroacetate or trichloroethanol: 7-10 μg/ml; DCVG: 8-10 μg/ml). This study provides evidence of the operation of the glutathione (reductive) pathway in humans. The markedly higher AUCs for the oxidative metabolites and absence of mercapturic acid conjugates in the urine of most volunteers supports the notion that the glutathione pathway is quantitatively minor for trichloroethylene. The failure to detect DCVC in the blood may be due to the unstable nature of this metabolite.

Green et al. (1998) proposed the hypothesis that formic acid excretion may have a role in trichloroethylene kidney toxicity and carcinogenesis. To test this hypothesis, groups of three or five male Fisher rats received single of repeated exposure for up to 28 days to trichloroethylene by gavage (1,000 mg/kg/day) or inhalation (250 or 500 ppm, 6 hr/day). Exceptionally high levels of formic acid were found in the urine of all trichloroethylene exposed rats. Excretion reached a peak 2 days after a single exposure, and reached a maximum after 4 days repeated exposure. Acute gavage administration of radiolabelled trichloroethylene showed that about 15% of the dose was excreted in the urine within 2 days, and about 86% of the radioactivity in the urine was

present as trichloroethanol glucuronide, suggesting that the formic acid was not a metabolite of trichloroethylene. Further investigations showed that the major metabolites trichloroethylene, trichloroethanol and trichloroacetic acid, also stimulated folic acid excretion (Dow and Green, 2000). Addition of folic acid to the diet or drinking water modulated the folic acid excretion response. Also, it was found that two markers of vitamin B12 deficiency, urinary methylmalonic acid and plasma 5-methyltetrahydrofolate were increased following repeated oral exposure to trichloroethanol. The authors postulated that trichloroacetic acid and trichloroethanol interact with vitamin B12 inducing a vitamin B12 deficiency which causes an inhibition of the B12 dependent methionine salvage pathway leading to a folate deficiency. As a result of the folate deficiency, the metabolic pathway that utilises formic acid is disrupted and excess formic acid is excreted in the urine. There is little information on whether trichloroethylene influences the methionine salvage pathway or formic acid excretion in humans.

Overall, two biologically plausible modes of action for trichloroethylene kidney toxicity and tumour formation have been proposed, but there remains considerable uncertainty as whether these operate in the rat, their relative importance to tumour formation, relevance to humans, and, indeed if other uninvestigated modes of action have a role.

Summary of proposed modes of action for trichloroethylene toxicity/carcinogenicity

Trichloroethylene has been shown to induce apparently species and strain specific toxicity in the mouse liver, the mouse lung and the rat kidney. In order to interpret the significance of these changes for human health, investigations have been carried out to elucidate the mechanisms involved. There is a growing body of evidence to show that development of liver tumours in mice is linked to the way mice metabolise trichloroethylene. In this species trichloroethylene is much more readily metabolised to trichloroacetic acid, a metabolite which has also been shown to induce peroxisome proliferation and to cause sustained cell proliferation in mice. It is thought that these effects in combination lead to the development of liver tumours in this species. Studies *in vitro* have shown that the metabolism of trichloroethylene in human hepatocytes is closer to that found in rat hepatocytes (i.e. much less than in mouse cells) and rat is a species which does not develop liver tumours following exposure to trichloroethylene. In addition, human hepatocytes have been shown not to undergo peroxisome proliferation in response to trichloroacetic acid whereas both mouse and rat hepatocytes do. It therefore seems reasonable to conclude that the findings in mice are unlikely to be of significance for humans.

There is also evidence linking the development of tumours in the mouse lung with metabolism of trichloroethylene in mouse Clara cells. It has been demonstrated *in vitro* that mouse Clara cells will metabolise trichloroethylene to chloral hydrate but are inefficient at detoxifying this metabolite. This means that chloral hydrate builds up within the Clara cell and it is thought that the build up of chloral hydrate within Clara cells results in cytotoxicity and repeated cycles of cell destruction and replication leading to tumour formation. In contrast, perfused rat lungs exposed to trichloroethylene *in vitro* did not accumulate chloral hydrate and human lung tissue appears to possess a negligible capability to metabolise trichloroethylene to chloral hydrate. This suggests that the lung tumours seen in mice are caused by a mode of action that is not relevant to humans.

The mode of action by which rats develop kidney toxicity and kidney tumours is less clear. There is evidence to show that hyaline droplet nephropathy is not involved. It has been suggested that the kidney tumours in rats arise as a result of repeated cytotoxicity. One mode of action that has been proposed involves metabolism via the glutathione conjugation pathway to form DCVC, which can be activated by renal β–lyase to reactive metabolites that are known to be mutagenic

and nephrotoxic. Species differences in the rates of glutathione conjugation and activation of DCVC by β-lyase have been identified, but these differences are not consistent with the known species differences in sensitivity to kidney toxicity. The presence of metabolites from the glutathione pathway have been detected in humans, but it seems that this is qualitatively a very minor pathway in all species, although this is not convincing evidence of a lack of toxicological significance. A second proposed mode of action involves a trichloroethylene induced increased excretion of formic acid, possibly resulting from an inhibition of the methionine salvage pathway. Whether this mode of action can operate in humans is not known. Given the uncertainty surrounding the mode of action by which nephrotoxicity occurs in rats and relevance to humans these findings are of concern for human health.

4.1.2.8.2 Human carcinogenicity

Cohort mortality studies

The health effects of occupational exposure to trichloroethylene have been investigated in a number of cohort studies in which the mortality (and sometimes morbidity) of a group of exposed workers is compared with that of the general population or, for one study, a matched control group.

In an extensive and carefully designed study, the mortality and cancer morbidity experiences of a group of 1,670 (1,421 men, 249 women) trichloroethylene exposed workers in Sweden were determined (Axelson et al., 1994). This study updated an earlier investigation (Axelson et al., 1978). The study cohort comprised of all workers who could be identified as having participated in a biomonitoring program (analysing urinary trichloroacetic acid levels) between 1955 and 1975. This service had been offered free to all Swedish customers of the country's sole producer of trichloroethylene. Cause of death and cancer morbidity were determined from Swedish registries, with a follow-up to 1987. For each individual, the level of trichloroethylene exposure was assessed on the basis of the mean urinary trichloroacetic acid concentration for all available samples and exposure time was calculated as the time between the first recorded urine sample and the termination of employment. The authors thought it likely that exposure was predominantly to trichloroethylene stabilised with diisopropylamine, although prior to 1975 there may have been some exposure to trichloroethylene containing epichlorohydrin and epoxybutane as stabilisers.

The majority of workers had mean urinary trichloroacetic acid levels (u-TCA) of less than 50 mg/l, which according to the authors, corresponds roughly to a time-weighted average exposure level of about 20 ppm. For male trichloroethylene workers the overall mortality was similar to the general population (standardised mortality ratio (SMR) 0.97, 95% CI 0.86-1.10). Male cancer deaths were less than expected (SMR 0.65, 95% CI 0.47-0.89), but mortality from diseases of the circulatory system was higher than expected, just achieving statistical significance (SMR 1.17, 95% CI 1.00-1.37). Mortality was also analysed after categorising workers according to exposure level (urinary trichloroacetic acid level less than 49, 50 - 99 or greater than 100 mg/l), exposure time (less than or greater than 2 years) and in a subgroup with a latency period of at least 10 years between first exposure and death; no excess deaths attributable to trichloroethylene exposure were seen in any subcohort. Overall cancer morbidity was similar to the general population among male trichloroethylene exposed workers (SIR 0.96, 95% CI 0.80-1.16). With regard to specific sites, a statistically significant increase was seen only for malignant skin tumours (8 observed cases, SIR 2.36, 95% CI 1.02-4.65); however, the excess occurred mainly in the low exposure group suggesting the skin cancers were not causally

associated with trichloroethylene exposure. There were also slight excesses of cancer of the liver (SIR 1.41, 95% CI 0.38-3.60), larynx (SIR 1.39, 95% CI 0.17-5.00), prostate (SIR 1.25, 95% CI 0.84-1.84) and of non-Hodgkin's lymphoma (SIR 1.51, 95% CI 0.51-3.64), but these were not statistically significant. For women, overall mortality was significantly higher than expected (SMR 1.55, 95% CI 1.02 - 2.31), due mainly to non-significant increases in deaths from circulatory disease and malignant tumours; the excess cancer risk was seen mainly among women with less than two years exposure. Cancer morbidity was not significantly increased among trichloroethylene exposed women (22 observed cases, SIR 1.32, 95% CI 0.53-3.79).

Overall, this study shows that there was no increased risk of cancer in men from trichloroethylene exposure at the levels encountered in Swedish industry. A reasonable degree of confidence can be attached to this outcome because the strength of the study design, in particular, the large number of subjects, consideration of morbidity as well as mortality and allowance of a possible latency period from first exposure. However, this study provides little useful information with respect to persons exposed to high levels (corresponding to u-TCA levels of >50 mg/l) or to women because of the small numbers of subjects in these subcohorts.

An earlier similar mortality study, based on a slightly larger group of individuals, has been carried out in Finland (Tola et al., 1980, cited in HSE, 1982). A total of 2,117 (1,148 men, 969 women) individuals were identified as being exposed to trichloroethylene at some time between 1963 and 1976, either from the records of the main laboratory in Finland responsible for urinary analysis of trichloroacetic acid (conducted as part of a mandatory health monitoring program) or from employer's reports. The vital status, or cause of death of members of the cohort was ascertained, as of November 1976, from national registries. The age-, sex- and cause-specific mortality rates for both the total cohort and a subcohort of those first exposed before 1970 were compared to that of the general population.

The highest urinary trichloroacetic acid level found in most (91%) workers was below 100 mg/l (which according to data of Axelson et al. (1994) indicates exposures of less than 40 ppm). The total number of deaths in the exposed cohort during the period under investigation was 58, compared with 84.3 expected (SMR 0.69). No statistical difference was noted in the age or sex specific overall mortality pattern, nor in cancer deaths, either in the total cohort, or in the sub-group exposed before 1970. There were eleven cancer deaths in the total cohort, as compared with 14.3 expected. These consisted of four cases of lung cancer (three men, one woman), three cases of cancer of the uterus, and single cases of other types of malignancies. A total of nine deaths due to malignancies were noted in those exposed prior to 1970, as opposed to 12.3 expected in this group. Thus, this study suggests that there is no increased risk of death from cancer or any other causes in the exposed group. However, this study has a major limitation in that the follow-up period may have been insufficient to detect a possible carcinogenic effect.

In a recent well-conducted and extensive study, the cancer experiences of a cohort of 1,698 male and 1,391 female workers exposed to trichloroethylene in Finland were investigated (Anttila et al., 1995). The cohort comprised of all workers in Finland who had participated in a mandatory biological monitoring program between 1965 and 1982, together with an additional 109 workers known to have suffered from trichloroethylene poisoning. This study included most of the individuals investigated in the Tola et al. (1980) study. The cancer incidence in the cohort, to the end of 1992, was determined by reference to a national cancer registry and compared with that of the general population. Comparisons were made for the total cohort and for subcohorts with u-TCA levels of less than or greater than 13 mg/l and latency periods (time from first u-TCA measurement to death or end of study) of 0 - 9, 10 - 19 or more than 20 years.

The mean latency period was about 18 years. Mean u-TCA levels for men were 6.3 mg/l and for women 8.3 mg/l. A total of 208 cancer cases were found in the study cohort, which was similar to the expected number (SIR 1.05, 95% CI 0.92-1.2). However, there was a significantly increased risk of cancer for the subcohort with a latency period of greater than 20 years (SIR 2.98 95% CI 1.20-6.13). Looking at specific sites, there were significantly increased risks at a number of sites; for cervical cancer (8 observed cases, SIR 2.42, 95% CI 1.05-4.77), with the risk being slightly increased in a subcohort with u-TCA greater than 13 mg/l (5 observed cases, SIR 4.4, 95% CI 1.4-10) and among those with the shortest latency period, of 0-9 years (6 observed cases, SIR 3.39, 95% CI 1.24-7.38); for liver cancer for men with a latency period of greater than 20 years (3 observed cases, SIR 13.0, 95% CI 2.68-37.9); for lymphohaematopoietic tissues, especially non-Hodgkin's lymphoma, among those with a latency period of greater than 20 years (7 observed cases, SIR 2.98, 95% CI 1.20-6.14); for the stomach among those with a latency period of greater than 20 years (7 observed cases, SIR 2.98, 95% CI 1.20-6.14); for prostate cancer among men with a latency period of greater that 20 years.

Overall, this is a robust study, involving a large number of subjects and with a long follow-up period. It provides some limited evidence of an increased risk of cancer among persons occupationally exposed to trichloroethylene. Other studies provide some supporting evidence for an association with an elevated risk of non-Hodgkin's lymphoma; there were slight, though not statistically significant, increases for non-Hodgkin's lymphoma in the Axelson et al. (1994), Blair et al. (1998) and Boice et al. (1999) studies. However, there is a lack of corroboration elsewhere for the findings relating to other cancer sites so, overall, no definitive conclusions about causality can be made.

The largest cohort mortality study involving trichloroethylene exposed workers was conducted in workers at a Utah (USA) aircraft maintenance facility (Spiritas et al., 1991; Stewart et al., 1991). This carefully-designed study was conducted as a response to concerns about an apparent excess of lymphatic and haemopoietic cancers in a small group of workers at the facility. The main study cohort comprised of all white persons who had been employed at the plant for at least one year between 1951 and 1956, totalling 14,006 persons. A subcohort of about 7,000 individuals who had been exposed to trichloroethylene, representing about 45,000 person years of exposure, was identified. The vital status of cohort members as of December 1982 was determined (ascertainment was 97%) and their mortality experience was compared to that of the general population of Utah.

A number of solvents had been used at the facility, primarily chlorinated hydrocarbons (carbon tetrachloride, chloroform, ortho-dichlorobenzene, freon, methylene chloride, perchloroethylene, 1,1,1-trichloroethane, trichloroethylene), aromatic hydrocarbons (toluene, xylene) and various alcohols; trichloroethylene was said to be the most widely used. Exposure assessments were conducted on the basis of information derived from a variety of sources, including process surveys, job descriptions, personnel files, employee interview and occupational hygiene data. Special efforts were made to evaluate trichloroethylene exposure. Although a quantitative determination of actual exposures could not be made, it was possible to develop exposure indices that reflected comparative exposures allowing the mortality data to be analysed by type of exposure (frequent vs. infrequent peak exposure, or continuous vs. intermittent low-level exposure), average exposure and cumulative exposure.

For the total cohort the SMR for all causes of death was significantly less than expected (SMR 0.92, 95% CI 0.90-0.95). Mortality was significantly increased for just three causes of death: cancer of miscellaneous lymphopoietic tissues (SMR 1.47, 95% CI 1.02-2.05), multiple myeloma (SMR 1.47, 95% 1.04-2.62) and asthma (SMR 1.47, 95% CI 1.00-2.62). Among the trichloroethylene exposed cohort, mortality from all causes was also significantly less than

expected for both men (SMR 0.92, 95% CI 0.87-0.96) and women (SMR 0.82, 95% CI 0.71-0.95) and there were no statistically significant excesses for cancer deaths or for any other specific cause of death. For males there was a significant upward trend for SMRs with cumulative exposure for all causes of death (SMRs for lowest, middle and highest exposure categories were 0.78, 0.88 and 0.99, respectively) and for emphysema (SMRs 0.31, 0.90 and 1.31, respectively) but no significant trends for cancer deaths were apparent. For women there were no noteworthy mortality trends with respect to cumulative exposure or for either sex when analysed on the basis of the type of exposure or average exposure.

The mortality experience of this cohort was followed up to the end of 1990 (Blair et al., 1998). Workers exposed to trichloroethylene showed non-significant increased relative risks for non-Hodgkin's lymphoma (RR 2.0, 95% CI 0.9-4.6) and cancers of the oesophagus (RR 5.6, 95% CI 0.7-44.5), colon (RR 1.4, 95% CI 0.8-2.4), primary liver (RR 1.7, 95% CI 0.2-16.2), breast (RR 1.8, 95% CI 0.9-3.3), cervix (RR 1.8, 95% CI 0.5-6.5), kidney (RR 1.6, 95% CI 0.5-5.1) and bone (RR 2.1, 95% CI 0.2-18.8). None of these cancers showed a relationship with exposure level. This, together with the absence of statistical significance, suggests that these differences were chance findings.

This study provides evidence that cancer mortality was not influenced by occupational exposure to trichloroethylene. The study involved large numbers of subjects, with a long follow-up period and high ascertainment rate, providing reassurance about the power of the study to detect an effect. A limitation of this study is that trichloroethylene exposure was based on assumptions rather than real monitoring data, raising the possibility of exposure misclassification, which could weaken the power of the study.

Mortality and cancer experience was ascertained in a group of 20,508 workers at an aircraft manufacturing plant in Arizona (Morgan et al., 1998). Comparisons were with the US national rates. The cohort comprised of all workers who were employed at the plant for at least 6 months between 1950 and 1985). Mortality was followed up to 1993. Exposure to trichloroethylene was assessed by hygienists, on the basis of job category; quantitative exposure data were not available. Information on possible confounding factors or on exposure to other chemicals was not collected. 23% of the cohort was considered to have had trichloroethylene exposure. For workers exposed to medium or high levels of trichloroethylene there were slight non-significant elevated risks for cancers of the kidney (RR 1.9, 95% CI 0.9-4.2), bladder (RR 1.4, 95% CI 0.5-3.8) and prostate (RR 1.5, 95% CI 0.9-2.6). Among women with medium and high peak exposure, the risk for ovarian cancer was slightly increased (RR 2.7, 95% CI 0.8-9.0) and for women with high cumulative exposure the risk for ovarian cancer was significantly increased (RR 7.1, 95% CI 2.1-23.5). The finding of a statistically significant difference at only one site suggests a chance occurrence. Overall, this study provides evidence of the absence of an association between trichloroethylene exposure and cancer. As with the previous study (Blair et al., 1998), this involved large numbers of subjects, with a long follow-up period and high ascertainment rate, providing reassurance about the power of the study to detect an effect.

In the most recent large cohort study, the cancer and mortality experience was ascertained in a group of 77,965 workers at an aircraft manufacturing plant in California (Boice et al., 1999). Comparisons were made with the US national rates. All workers who had been employed for more than one year after the start of 1960 were included. Vital status at the end of 1996 was determined. Exposure to trichloroethylene and other chemicals was assessed on the basis of job title. The number of workers exposed to trichloroethylene was 2,267, many of whom were also exposed to tetrachloroethylene, a variety of other solvents, and chromate. The overall risk for all cancers or cancers at specific sites was not increased for workers with trichloroethylene exposure.

However, for workers exposed to trichloroethylene for more than 5 years, there was a non-statistically significant increased risk for non-Hodgkin's lymphoma (RR 1.62, 95% CI 0.82-3.22).

The possible association between trichloroethylene exposure and renal cell cancer was investigated in cardboard factory workers in the Federal Republic of Germany (Henschler et al., 1995). The study cohort comprised of all workers in a factory who had been exposed to trichloroethylene for at least one year between 1956 and 1975; the mean duration of exposure was about 18 years. Of the 183 (all male) eligible workers morbidity and mortality data to the end of 1992 were obtained for 169 workers. The levels of trichloroethylene exposure in the factory had not been monitored, but interviews with employees and a review of working practices suggested that the workers had been exposed repeatedly or continuously to significant levels of trichloroethylene during the study period. Company records indicated that insignificant amounts of other solvents were used in the factory at this time. A control group of 190 male workers from the same factory, who were not exposed to trichloroethylene, matched for age and physical job activity, were used for comparison. For living subjects medical and other records were consulted and physical examinations, including abdominal sonography, were conducted. For dead subjects, the causes of death were obtained from hospital or physicians records and from interviews with relatives. Additional comparisons were made with data from the cancer registries of Denmark and the former German Democratic Republic (the Federal Republic does not have a cancer registry) and mortality was compared with that of the local population.

There were no significant differences between the exposed and control groups with respect to body mass index, blood pressure, intake of diuretics, and smoking and drinking habits. Five cases of kidney cancer (four renal cell tumours and one urothelial cancer of the renal pelvis) were found among the exposed workers, compared with none in the control group; this difference was statistically significant. All cancers were detected at least 18 years after the start of exposure. Additionally, two further cases of kidney cancer in the exposed group were diagnosed in 1993, after the close of the study. Significantly elevated standardised incidence ratios (SIR) for renal cancer were calculated with reference to both the Danish and German Democratic Republic data; these were 7.97 (95% CI 2.59-8.59) and 9.66 (95% CI 3.14-22.55). Mortality for the exposed workers was similar to that of the control group and less than expected in comparison with the local population (standard mortality ratio (SMR) 0.68, 95% CI 0.48-0.93).

There are several major limitations to this study. Firstly, it is apparent that this study is a cluster investigation (i.e. the study was an investigation of cancer cases that had already been observed prior to the instigation of the study), and not a retrospective cohort study as claimed by the authors. This reduces somewhat the significance that can be attached to the result; cluster investigations are generally regarded as merely hypothesis-generating and should be followed up by independent hypothesis-testing studies conducted in other exposed populations. Secondly, there are doubts regarding the validity of the comparison with the general population, since detailed diagnostic procedures, including abdominal sonography, were conducted on the exposed subjects. Other limitations are that a relatively modest number of individuals were studied and actual trichloroethylene exposure levels could not be quantified. Overall, this study raises concerns about an association between trichloroethylene exposure and renal cancer, but because of the limitations relating to the conduct and design of this study it can only be considered as a hypothesis-generating study. It is noted that this study was followed up by a case-control study (Vamvakas et al., 1998), described in the following section, which also provided evidence of an association between trichloroethylene exposure and kidney cancer.

A retrospective cohort mortality study was conducted among 14,067 men and women who had been employed for 4 or more years between 1958 and 1982 at a US aircraft manufacturing company (Garabrant et al., 1988). Vital status at the end of 1982 was determined and the

mortality experiences were compared with national and local mortality data. Only very limited information was available on chemical exposure; interviews with 362 subjects indicated that a variety of chemicals were used in the plant, with around a third of jobs involving trichloroethylene exposure. SMRs for all causes of death and for cancer deaths were low and there were no significant excess cancers for specific sites. However, because chemical exposures were inadequately characterised, this study does not provide any useful information about the effects of trichloroethylene exposure.

The mortality experiences of workers at a US manufacturing plant in which trichloroethylene was used as a degreasing agent were studied (Shindell and Ulrich, 1985). All persons who had worked at the plant for more than 3 months from when the plant was opened in 1957 to 1983 were included in the study. The vital status of 2,646 (2,140 white males, 76 non-white males and 430 women) workers was determined, which represented about 98% of all employees. No information on the exposure levels to trichloroethylene was presented. Mortality of the study cohort was compared to that of the general population. For all three sex/race groups overall mortality (SMR for white males 0.79) and cancer mortality (SMR for white males 0.62) was less than expected. The value of this study is severely limited because the trichloroethylene exposures were not characterised and it is likely that persons with little or no exposure to trichloroethylene, such as office staff, were included in the study.

A retrospective mortality study was conducted on a group of 65 dry cleaning workers in Prague (Málek et al., 1979). The selection methods were not reported, but it was suggested that this group represented all men who had spent at least one year working in this industry in Prague since the 1950's. The exposure history to trichloroethylene and mortality experience of 57 of the men was determined. About 60% of the workers had been exposed for at least 5 years, and for almost 50% the time period from initial exposure to the time of the study was more than 20 years. An indication of exposure levels was available from the results of 408 urinary trichloroacetic acid determinations conducted on the group; these suggested that exposures were relatively high, with many instances of concentrations being greater than 100 mg/l. The contention that exposure levels were very high was supported by the reporting of 32 cases of trichloroethylene poisoning among trichloroethylene workers during the study period. Eleven workers died during the study period, six as a result of cancers (three of the lung, one rectal, one rectal and bladder and one tongue), all occurring in subjects aged 58 or over. The incidence of lung and rectal cancer was said to be roughly similar to that of the general Czechoslovakian population of comparable age. The results of this study suggest that occupational exposure to trichloroethylene, possibly at relatively high levels, is not associated with an increased cancer risk, but little weight can be given to these findings because of the small cohort size and lack of detail in the study report.

Case control studies

As a follow-up to the cohort study of Henschler et al. (1995), a case control study was conducted in Germany to assess whether high, prolonged, exposure to trichloroethylene can cause renal cell cancer (Vamvakas et al., 1998). A group of 73 cases of histologically confirmed renal cell cancers originating from the tubule epithelium were identified from patients attending a urology department of a country hospital in North Rhine Westphalia between December 1987 and May 1992. The cases were different from those of the Henschler et al. (1995) study. Eighty-four controls, unmatched to the cases, were patients attending the accident wards of three different hospitals, all located within 20 km of the hospital with a urology department, during 1993. Physicians using a specially designed questionnaire obtained information on occupational history, including exposures to hazardous chemicals. The physicians appeared not to be blind to

exposure status. Information on trichloroethylene and tetrachloroethylene exposure was obtained from occupational hygienists contracted to the employer's liability insurance association and from investigations relating to legal compensation cases involving occupational diseases claimed to be caused by trichloroethylene exposure. Exposures to trichloroethylene and tetrachloroethylene were ranked semi-quantitatively into high, medium and low exposure by applying a system that integrates total exposure time and frequency and severity of acute symptoms. The cases were statistically significantly older (mean age cases: 62 years, controls 51 years), heavier, had higher systolic and diastolic blood pressure, and higher frequency and duration of diuretic treatment. There were no differences with respect to sex ratio, body mass index, smoking habits and alcohol consumption. The odds ratio was significantly greater for trichloroethylene exposure versus no exposure when adjusted for age, gender, smoking, blood pressure and intake of diuretics (OR 10.8, 95% CI 3.4-34.8, 19 cases and 5 controls exposed).

There are major problems with the conduct of this study. There is high probability that the results were affected by bias. The interviews of the cases and the controls were carried out by physicians who were aware of case status and would have had knowledge of the hypothesis being investigated. Interviews for cases and controls should ideally be conducted by the same (trained) interviewer(s) who are unaware of case status. There is a strong possibility that cases' physicians would probe more deeply and cases would over-report exposures to trichloroethylene. This problem is exacerbated by the admittance that some of the cases are the subject of personal injury litigation. Bias will also be introduced because the method of selection of controls would exclude potential controls who would have been eligible for participation because they lived in the catchment area during the time period of case selection but had subsequently left the area or died. Additionally, it is not clear from the report and subsequent discussions with the authors that the control subjects, who were recruited from different hospitals, were from exactly the same catchment area as the cases. Thus, it is not possible to judge if the controls had the same opportunity for trichloroethylene exposure as the cases. Another problem is the age difference between the cases. The cases were on average 11 years older the controls and therefore the cases would have had more opportunity for trichloroethylene exposure. Additionally, changes in employment patterns would result in differences in the opportunity for trichloroethylene exposure between cases and controls. Overall, this study is considered to have major methodological flaws and is consequently given little weight in the overall assessment.

In connection with the above studies, several investigations were conducted in relation to a hypothesis that the renal cell cancers were related to metabolism of trichloroethylene via the glutathione conjugation pathway to form DCVC, which can be activated by renal β–lyase to reactive metabolites that are known to be mutagenic and nephrotoxic. Genetic phenotypes for two polymorphic isoenzymes of glutathione transferase, GSTM1 and GSTT1, were determined in a group of 45 cancer cell patients with a history of occupational exposure to trichloroethylene (Brüning et al., 1997). For comparison, a control of 48 persons of similar age with occupational exposure to trichloroethylene but without renal cell cancer was recruited. Based on information about clinical symptoms obtained by questionnaire, trichloroethylene exposures for both groups were thought to be several fold higher than 50 ppm. Mean duration of exposure was 18.1 years for the cancer patients and 15.7 years for the controls. The renal cell cancer patients were significantly more likely than controls to have at least one functional GSTM1 gene (OR 2.7, 95% CI 1.18-6.33) and at least one functional GSTT1 gene (OR 4.2 (95% CI 1.16-14.91). This suggests that the cancer patients were more likely to have a genotype indicating an ability to better metabolise trichloroethylene to a reactive species such as DCVC. Two studies looking for evidence of kidney damage in trichloroethylene workers are summarised in Section 4.1.2.6.2 (Brüning et al., 1996; Brüning et al., 1999). These studies revealed some interesting patterns of protein excretion in trichloroethylene exposed workers, but did not provide clear evidence of

trichloroethylene-related kidney damage. While the findings of these three studies are consistent with the hypothesis, they do not provide strong evidence to support the hypothesis.

In a population based case-control study in Minnesota, 438 histologically confirmed renal cell cancer cases were interviewed for occupational history (Dosemeci et al., 1999). The renal cell cancer cases were identified from the Minnesota State cancer registry as new diagnoses from mid-1988 to end of 1990. There were 687 controls, selected as an age and gender stratified random sample, presumably from the local Minnesota population. Occupational solvent exposure was estimated using exposure matrices based on job title. The analysis included adjustments for age, smoking, hypertension status and body mass index. The risk of renal cell carcinoma was significantly elevated for women exposed to all organic solvents combined (OR 2.3, 95% CI 1.3-4.2), to all chlorinated aliphatic hydrocarbons combined (OR 2.1, 95% CI 1.1-3.9) and to trichloroethylene (OR 2.0, 95% CI 1.0-4.0). No excess risk associated with solvent exposure was found among men. A major weakness in this study is the reliability of the exposure assessment, without more precise information it is not possible to draw firm conclusions regarding the possible association between trichloroethylene and renal cell cancer in females.

Other studies

The possible role of occupational exposure to trichloroethylene and five other chlorinated aliphatic hydrocarbons in the development of astrocytic brain cancer was investigated in a well-conducted case-control study (Heineman et al., 1994). Exposure indices, determined by occupational hygienists on the basis of occupational information obtained from questionnaires administered to next-of-kin, for 300 cases were compared with those of 320 controls, matched for sex, age, year of death and study area. Trichloroethylene exposure was not found to be a risk factor for astrocytic brain cancer. This study provides only limited reassurance of an absence of carcinogenic potential as only one type of cancer was studied and, because exposure assessments were based on next-of-kin questionnaire, the potential for misclassification was high.

The possible association between liver cancer and occupational exposure to trichloroethylene was investigated among cancer sufferers in Prague (Novotna et al., 1979, cited in HSE, 1982). At the time of the study it was estimated that there were about 550 persons occupationally exposed to trichloroethylene in the Prague area. A total of 63 cases of histologically confirmed cases of cancer of the liver were identified from Government records for the years 1972 and 1974. The investigators were able to obtain some information on the employment history of 56 of the patients (39 men, average age 69 and 17 women, average age 56). There was no evidence that any of the cases had ever been exposed to trichloroethylene within the previous 17 years. The limitations of this study are that it involved relatively small numbers of persons, only one type of cancer was studied and it is difficult to ascertain the reliability of the employment history. Consequently little weight can be given to these results.

The incidence of liver cancer among past employees of a UK trichloroethylene manufacturer was investigated using the regional cancer registry (Paddle, 1983). No information on exposure at the manufacturing plant was provided, although it was suggested that exposures would have been lower than in the trichloroethylene user industries. A total of 95 cases of liver cancer for the period 1951 to 1977 were identified from the registry. None of these cases were found to have been employed by the manufacturer between 1934 and 1976. Because of the lack of exposure data and because only one type of cancer was looked at, this study, while providing reassurance to this particular manufacturer about the risks to its workforce, the study contributes only limited information to the broader assessment of the carcinogenic potential of trichloroethylene.

The risk factors associated with colon cancer were investigated in a case-control study (Fredrickson et al., 1989). The cases consisted of all living persons aged 30-75 years with large bowel cancer adenocarcinoma who had been reported to the Swedish cancer registry between 1980 and 1983. A total of 156 male and 156 female cases (about 95% of all cases) agreed to participate in the study. A control group consisted of 306 males and 317 females, age- and sex-matched. Information on occupation, exposures, medical history, drug intake and smoking, drinking and eating habits were obtained by mailed questionnaire. The odds ratio for dry cleaners exposed to trichloroethylene was significantly increased (OR 7.4, 95% CI 1.1-47.0), although the odds ratios were not significantly increased for all dry cleaners (OR 2.0, 95% CI 0.5-7.1) or for all exposed to trichloroethylene (OR 1.5, 95% CI 0.4-5.7). There is, however, insufficient evidence to suggest that there is a causal association between trichloroethylene exposure and bowel cancer among dry cleaners for several reasons. The number of dry cleaners in the study was too small (5 cases and 5 controls) to form the basis for any firm conclusions, the exposure data were obtained by an unreliable method and there are no supporting data for this association from other studies.

A number of other studies, including some case control studies and community-based studies in populations exposed to drinking water contamination, are available and have been summarised by IARC (1995). These studies either provide little information specifically relating to trichloroethylene, or have methodological problems that complicate their interpretation and, set against the more informative cohort studies in occupational settings, provide no useful additional information on the carcinogenic properties of trichloroethylene. Consequently these studies are not considered in this risk assessment.

4.1.2.8.3 Summary of carcinogenicity studies

The carcinogenicity of trichloroethylene has been investigated in a number of long-term animal studies, using the oral and inhalation routes, and involving hamsters and a variety of strains of rat and mouse. These studies provide clear evidence that trichloroethylene is carcinogenic in rats and mice. In the mouse, trichloroethylene induced hepatocellular tumours by either the inhalation or oral routes and lung tumours by the inhalation route. The hepatocellular tumours were observed at high oral doses levels of 1,000 mg/kg/day and above (lower exposure levels were not investigated) and by inhalation, at 600 ppm, but not at 300 pm. The lung tumours occurred at exposure levels as low as 150 ppm in one study but not at 100 ppm in another. In the rat, trichloroethylene induced renal tubular adenomas, albeit at a low incidence, in association with other pathological changes. Kidney tumours were induced at an exposure level of 600 ppm but not at 300 ppm inhalation exposure; by oral exposure kidney tumours were seen at 500 mg/kg/day and above in some studies but not at 250 g/kg/day in another.

Since there are uncertainties regarding the role of genotoxicity in trichloroethylene induced carcinogenicity, other mechanisms of tumour formation, and their possible relevance to humans, should be considered. With regard to the liver tumours seen in mice, there is evidence that these are associated with the phenomenon of peroxisome proliferation, produced by the metabolite trichloroacetic acid. The extent of formation of this metabolite is much greater in mice than in rats, a species that does not develop liver tumours following trichloroethylene exposure, and in humans. Furthermore, humans are much less sensitive to the induction of peroxisome proliferation, compared with rodents. Accordingly, it is considered that the induction of liver tumours in mice is unlikely to be of relevance to human health. Turning to the lung tumours seen in mice, there is evidence to suggest that these may be associated with the accumulation of the metabolite chloral hydrate in Clara cells and that the accumulation of this metabolite is unlikely

to occur in humans. There is also uncertainty about the mechanism by which the kidney tumours arise in rats. There is no evidence of hyaline droplet nephropathy, a mechanism associated with male rat-specific kidney tumour formation. One possibility is that the kidney tumours are related to repeated cycles of cytotoxicity and proliferation, perhaps related to β-lyase activation of the trichloroethylene metabolite DCVC or to excess formic acid excretion, but little is known about the toxicity of trichloroethylene to the kidney in humans so it is not possible to judge the relevance of these tumours to human health.

The carcinogenicity of trichloroethylene has also been investigated in occupationally exposed populations in a number of studies. The majority of these did not show any evidence that trichloroethylene exposure is associated with an increased incidence cancer. Individually, each of the negative studies had certain limitations although several (Axelson et al., 1994; Spiritas et al., 1991; Blair et al., 1998; Morgan et al., 1998; Boice et al., 1999), by nature of their design, had substantial power to detect an effect, and when taken together provide significant evidence that trichloroethylene is not carcinogenic in humans under the exposure conditions experienced by the groups studied. However, the occupational studies did not provide complete reassurance of the absence of trichloroethylene-related carcinogenicity in humans. This was because limited evidence of an increased risk of cancer, in particular non-Hodgkin's lymphoma, among trichloroethylene exposed workers was reported in one well-conducted cohort study (Anttila et al., 1995), although definitive conclusions about causality could not be made in view of the limited corroboration with the results of the other studies. Furthermore, in two other studies an increased risk of kidney cancer was reported in groups of trichloroethylene workers, possibly experiencing higher exposures that subjects in the previously mentioned studies (Henschler et al., 1995; Vamvakas et al., 1998). The lack of corroboration between these two studies and the other epidemiology studies may be because of the differences in trichloroethylene exposure levels. However, it was not possible to draw firm conclusions from the Henschler et al. (1995) and Vamvakas et al. (1998) studies because of methodological weaknesses, but the reported association does add to the concerns about the carcinogenic potential of trichloroethylene.

Overall, given the observation of kidney tumours in animal studies and absence of convincing evidence that these arose by modes of action not applicable to humans, together with the uncertainties regarding the human data, it is considered that there remains some concern for potential carcinogenicity in humans.

There have been uncertainties about whether trichloroethylene should be classified for potential carcinogenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity.

The Specialised Experts were concerned about observations of kidney tumours and non-Hodgkin's lymphoma in humans. They concluded that the elevation of kidney tumour morbidity in two epidemiological investigations in Germany (Henschler et al., 1995; Vamvakas et al., 1998) indicates an association between exposure to high airborne concentrations of trichloroethylene and kidney cancer formation in humans. Consistent with these findings, some of the Specialised Experts drew attention to an increase in the relative kidney cancer risk observed in the large retrospective cohort study of workers in aircraft maintenance (Blair et al., 1998). Nevertheless, the evidence was not considered sufficient to clearly establish a causal association to trichloroethylene exposure, as bias could not be completely ruled out. The Specialised Experts also agreed that several cohort investigations (Axelson et al., 1994; Anttila et al., 1995; Blair et al., 1998; Boice et al., 1999) show an association between exposure to trichloroethylene and development of non-Hodgkin's lymphoma, but bias and confounders could not be completely ruled out. With the exception of one, all Specialised Experts considered that the evidence did not meet the criteria for classification as a category 1 carcinogen. One Expert,

in support of category 1, underlined the evidence for kidney tumours in humans and the consistency with the *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) metabolic pathway and the observation of a different spectrum of somatic mutations in kidney tumours of trichloroethylene-exposed compared to unexposed patients.

A clear majority of the Specialised Experts recommended that classification of trichloroethylene as a category 2 carcinogen is warranted, based on evidence in one animal species, namely tumours in the rat kidney, supported by epidemiological data showing an association between exposure and kidney tumours and non-Hodgkin's lymphoma in humans. Some Specialised Experts stated that genotoxicity and metabolic/biochemical findings added to their concern. One expert maintained that category 1 was appropriate, one preferred category 3 but accepted the majority view.

The Specialised Experts considered four plausible, not mutually exclusive mechanisms for kidney tumour formation possibly relevant for humans, but agreed that there was insufficient evidence for any of them to be considered as proven. One mechanism involves the formation of reactive intermediates locally in the kidney by beta lyase following metabolism of trichloroethylene via a glutathione pathway. A second mechanism involves renal toxicity via the accumulation of formic acid and, potentially, a perturbation of the methylation status. The other two plausible mechanisms involved genotoxicity. The Specialised Experts considered that trichloroethylene might pose a carcinogenic hazard through either a pathway involving induction of aneuploidy, or one involving mitotic recombination or point mutations.

In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly. This conclusion has been carried forward to the risk characterisation.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Fertility and general reproductive performance

Inhalation

The effects of inhalation exposure on fertility and reproductive performance have not been investigated. However, in two studies the effects on sperm morphology have been investigated.

Groups of twelve Sprague-Dawley rats and twelve CD mice were exposed to trichloroethylene (purity 99.9%) at concentrations of 0, 100 or 500 ppm, 7 hours/day for 5 consecutive days (NIOSH, 1980). Groups of four animals were killed at 1, 4 or 10 weeks after dosing and sperm was sampled. In rats, trichloroethylene had no effect on the proportion of morphologically abnormal sperm; unfortunately there was also no response in a positive control group (TEM administered by the intraperitoneal route). In contrast, for mice there was a statistically significant increase in the frequency of abnormal sperm at the highest exposure level in weeks 1 and 4 (negative control and high-dose group mean percentage abnormalities were 6.8 and 18.9 for week 1 and 8.1 and 23.5 for week 4) and at the lower level in week 4 (mean percentage 14.3). An appropriate response occurred in a positive control group. It is noted that in another sperm morphology study (testing perchloroethylene) described in this report, the percentage of

abnormal sperm in the control group on day 10 was 13.0%, indicating that the background incidence of sperm abnormalities is very variable. It the light of this observation, it is reasonable to consider the possibility that the frequency reported for the low-dose group could be due to chance.

In the second study, mice (C57B1OxC3H strain) were exposed to trichloroethylene at concentrations of 0, 200 and 2,000 ppm, 4 hours/day for 5 days; the number of animals in each group was 15, 5 and 10, respectively (Land et al., 1981). Sperm were sampled from the cauda epididymides 28 days after the first exposure. The percentage of abnormal sperm in animals from the 2,000 ppm group (2.43 ± 0.15) was significantly greater than observed in the control group (1.42 ± 0.08). At 200 ppm the percentage (1.68 ± 0.17) was similar to the control value.

The two sperm abnormality studies provide evidence that, in mice, short-term trichloroethylene exposure by the inhalation route can affect sperm morphology. The second study indicates that the NOAEL for this effect is 200 ppm. However, similar effects have not been observed in any rat study, or in mice receiving relatively high doses in large-scale long-term oral studies (see following section).

Oral

The effects of trichloroethylene on fertility and reproductive performance have been extensively studied in mice and rats using the test system known as the "Reproductive Assessment by Continuous Breeding" (NTP, 1986). This system involves four successive tasks. Task 1 is a preliminary 14-day toxicity study, conducted so that appropriate dose levels for the subsequent tasks can be selected. Task 2, the continuous breeding phase, involves a 14-week cohabiting phase during which reproductive performance is monitored. In Task 3, an optional "cross-over" mating trial is conducted; control males are mated with high-dose females and high-dose males are mated with control females. This is to determine whether any adverse effect seen in Task 2 is mediated through males of females. In Task 4 the reproductive performance of the F1 offspring taken from the Task 2 final litters is assessed. The test substance is administered continuously through Tasks 2, 3 and 4 (except during the Task 3 mating phase).

The first study was conducted in CD-1 mice. The test substance ("Hi-Tri" purity grade trichloroethylene) was microencapsulated in a gelatin/sorbitol shell and administered by incorporation in the diet. Groups of twenty males and twenty females (F0 generation) were continuously exposed to the test substance at concentrations of 0.15, 0.3 or 0.6% (resulting in dose levels of approximately 187, 350 or 750 mg/kg/day) during a one week premating period and 17 week mating trial (Task 2). The dose levels were selected on the basis of a preliminary study (Task 1), in which significant reductions in bodyweight gain were seen at dietary trichloroethylene concentrations of 1.2% and above. The control group comprised of forty animals of each sex. After the premating period, males and females from each group were randomly paired and allowed to cohabit for 14 weeks. During the cohabiting period the reproductive performance was monitored by counting the number of F1 generation litters produced by each breeding pair and recording on the day of birth the litter size, proportion of live pups, litter weight and sex ratio of the pups; all pups were then immediately removed and discarded. All litters produced after the cohabiting period remained with their mothers until weaning to day 21 post partum. Twenty male and twenty female F1 generation offspring from the control and high-dose groups were retained after weaning for assessment of their reproductive capacity (Task 4). After rearing to sexual maturity, each F1 female was paired with a F1 male from the same dose group for seven days. The resulting litters were evaluated and discarded on the day of birth as described for the litters produced during the F0 generation cohabiting phase. For all control and high-dose F0 and reared F1 animals, liver, kidneys,

adrenals and reproductive organs were weighed and subjected to histopathological examination and for males sperm analysis (concentration, motility and morphology) was undertaken. Task 3 was not conducted.

There were no clinical signs of toxicity or adverse effects on bodyweight gain among F0 generation animals. Trichloroethylene exposure had no effect on fertility, with the number of litters produced and litter sizes being similar for the control and treated groups. However, F1 pup bodyweight at birth and, for final litters during lactation, was slightly, though statistically significantly, reduced at the highest dose level in comparison with controls. Also, the proportion of final litter pups dying during lactation at this dose level was greater than observed in the control group (61% compared with 28%), although the proportion of live pups at birth was similar. Fertility of the reared F1 generation was not affected by treatment.

At necropsy of the F0 generation, treatment-related effects on liver weight and sperm motility were seen at the highest dose level; bodyweight-adjusted liver weight for both sexes was increased by 30-40% relative to controls and the proportion of motile sperm was 43% compared with 78% in the control group. Additionally, histopathological changes were reported in the liver and kidney; these included hypertrophy of the centrilobular liver cells and renal tubular degeneration and karyomegaly of the tubular cells. Similar organ weight, sperm and histopathological changes were noted at the highest dose level in the reared F1 generation.

This study showed that long-term administration of trichloroethylene to the mouse at 750 mg/kg/day, an exposure level at which other toxic effects are elicited, can influence the reproductive process, causing reduced sperm motility and slight reductions in neonatal bodyweight and survival. A NOAEL for these effects was 350 mg/kg/day.

The second study was conducted in F344 rats (NTP, 1986), using the same dietary levels as the mouse study (resulting in estimated dose levels of approximately 75, 150 and 300 mg/kg/day). The protocol was identical, with the exceptions that Task 3 was conducted, in Task 4 the reproductive capacity of pups taken from all four experimental groups was assessed and the open field behaviour of the F1 generation was evaluated at 21 and 45 days of age.

There was some evidence of generalised toxicity in the F0 generation, observed as a slight reduction in bodyweight gain for both males and females at all treatment levels, although for males a clear dose-response relationship was not apparent. A similar reduction in bodyweight gain, which followed a clear dose-related pattern, was seen in the retained F1 generation animals of both sexes at all treatment levels.

All treated F0 generation animals were fertile. However, there was a slight, but statistically significant, reduction in the number of litters produced by high-dose F0 pairs (mean 2.90 ± SD 0.22 compared with 3.49 ± 0.15 for control) and a decrease in the litter size in the middle and high-dose groups (9.39 ± 0.35 and 8.66 ± 0.64, respectively compared with 10.36 ± 0.36 for the control). At the F0 generation cross-over mating trial (Task 3), there were no treatment-related effects on the fertility of either males and females from the high-dose group or on the numbers of pups in the resultant litters. The reproductive capacity of the reared F1 generation was not affected by trichloroethylene treatment.

At the necropsy of the F0 generation (conducted only for control and high-dose animals) bodyweight adjusted liver mean weight was significantly increased for both sexes (by about 20% relative to controls) and combined left testes/epididymis mean weight was increased (by about 3%) in the high-dose group. Also, among females only, combined kidney/adrenal weight was increased (by about 7%). However, there were no effects on semen quality or treatment-related histopathological changes. For the F1 generation, the only organ change considered to be

treatment-related was increased bodyweight-related liver weight in males at all treatment levels (by 16% at the highest dose) and in females in the middle and high-dose groups (by about 10% at the highest dose).

This study showed that long-term trichloroethylene exposure to the rat can have an effect on reproduction, causing reductions in the number of litters born to continuously bred animals at 300 mg/kg/day and in the litter size at 150 and 300 mg/kg/day, dose levels at which other toxic effects were elicited. No effects were seen at 75 mg/kg/day.

The effects of trichloroethylene on female fertility have been investigated in a comprehensive study (Manson et al., 1984). Groups of 23 female rats (Long-Evans hooded) received gavage doses of trichloroethylene (electronic grade, >99.9% pure) in a corn oil vehicle at levels of 0, 10, 100 and 1,000 mg/kg/day for 2 weeks prior to mating, during a mating period, and throughout pregnancy. The females were dosed 5 days/week during the premating and mating periods and 7 days/week during pregnancy. The mothers were allowed to litter and were killed on day 21 post partum. Pup survival and bodyweight gain was monitored until day 31 post partum. Maternal bodyweight gain during the premating period and pregnancy was significantly reduced at the highest dose level. No effects on female fertility, oestrus cycling, litter size or pup bodyweight gain were observed. However, pup survival to weaning at the highest dose level was significantly reduced (83% survival compared with 92% in the control group).

The effects of trichloroethylene exposure on certain aspects of male reproductive function were investigated in groups of ten Long-Evans rats (Zenick et al., 1984). Trichloroethylene (electronic grade, purity >99.9%) was administered in a corn oil vehicle at levels of 0, 10, 100 or 1,000 mg/kg/day, 5 days/week for six weeks, followed by a four-week recovery period. At 1, 5 and 10 weeks, copulatory behaviour (with untreated females) was assessed and sperm samples were analysed for sperm count, motility and morphology. Additionally, plasma testosterone levels were analysed at weeks 6 and 10. General toxicity occurred only at 1,000 mg/kg/day, observed as reduced bodyweight gain and increased liver weight. Treatment-related effects on copulatory behaviour were observed at 1,000 mg/kg/day, only at week 1; the males neglected the females for long periods and there were numerous instances of incomplete genital contact. The authors suggested that this effect may have been due to the narcotic properties of trichloroethylene. The sperm parameters and testosterone levels were not affected by trichloroethylene exposure at any treatment level. The influence of trichloroethylene on copulation was confirmed in a subsequent experiment, in which increased ejaculation latency was seen following oral administration at a level of 1,000 mg/kg (Nelson and Zenick, 1986). Naltrexone, an opiate antagonist, blocked this effect, suggesting that the effect of trichloroethylene may be mediated through endogenous opiate system.

Summary of fertility and general reproductive performance

Considering inhalation exposure, the effects on only one parameter that relates to reproduction have been investigated. Two studies in mice have suggested that short-term repeated trichloroethylene exposure can influence sperm morphology; a NOAEL for this effect was 200 ppm. However, effects on sperm morphology were not seen in rats, nor in large-scale studies in which mice received long-term exposure to high levels of trichloroethylene by the oral route.

The effects of long-term oral administration on fertility and reproductive performance have been extensively investigated in rats and mice. Trichloroethylene was shown to have some influences on reproduction and only at exposure levels that produce general toxicity. The observed effects included reduced sperm motility and reductions in neonatal bodyweight and survival in mice at high-dose levels and, in rats, disrupted copulatory behaviour and reduced pup survival at high-

dose levels, and slight reductions in the litter size and number of litters born to continuously bred animals. NOAELs for reproductive effects were 350 mg/kg/day in mice and 75 mg/kg/day in rats.

Developmental toxicity

Inhalation

Groups of mated female mice (Swiss Webster) and rats (Sprague-Dawley) were exposed to trichloroethylene (NEU-TRI®, 99.2% pure, probably with epichlorohydrin added as a stabiliser) vapour at airborne concentrations of 0 or 300 ppm for 7 hours/day from day 6 to 15 of pregnancy (Schwetz et al., 1975) The numbers of pregnant females in each group were twenty-six control and twelve treated mice and thirty control and eighteen treated rats. The females were killed on day 18 (mice) or 21 (rats) and the uterine contents were examined. The foetuses were weighed, measured and subjected to detailed external, visceral and skeletal examinations. No evidence of maternal or developmental toxicity was observed. However, this study cannot be considered a thorough evaluation of the developmental toxicity of trichloroethylene because only a single dose level, which did not elicit maternal toxicity, was used and the group sizes were less than recommended in internationally recognised guidelines.

Groups of 30 female rats (Long Evans hooded) were exposed to trichloroethylene (NEU-TRI®, 99% pure, with 0.2% epichlorohydrin) vapour at a concentration of 1,800 ppm, either for two weeks prior to mating and throughout pregnancy, or for two weeks prior to mating alone or during pregnancy alone (Dorfmueller et al., 1979). A similar control group received filtered air under identical conditions prior to mating and during pregnancy. Half of the mothers from each group were killed on day 21 of pregnancy and the foetuses were weighed and examined for external, visceral and skeletal abnormalities. The remaining mothers were allowed to litter and offspring were retained until up to an age of 100 days. The activity levels of the offspring were assessed at 10 days of age (four pups per litter) and at 20 and 100 days of age (one male and one female per litter) using automated electronic methods. No evidence of maternal toxicity was observed. Litter size, post-implantation loss, foetal weight and postnatal activity levels were similar for all groups. The foetal examination revealed a slight increase in the incidence of minor visceral and skeletal abnormalities in the group with exposure during pregnancy only, but in the absence of similar findings in the group with premating and pregnancy exposure these findings should not be attributed to trichloroethylene exposure. Also, the bodyweight gain of retained pups from both groups exposed prior to pregnancy was slightly reduced from 20 to 100 days of age in comparison with the other two groups, but this finding is considered more likely to have arisen by chance than to be caused by maternal exposure prior to pregnancy. Overall, this study provided no convincing evidence of developmental toxicity following maternal trichloroethylene exposure either before or during pregnancy, although the value of this study is limited by the use of a non-maternally toxic dose level.

In another study, groups of female rats (Sprague-Dawley) and rabbits (New Zealand White) were exposed to trichloroethylene (purity 99.9%) vapour at a concentration of 0 or 500 ppm for 7 hours/day during pregnancy (NIOSH, 1980). For the rats, 20 -28 females were allocated to each group, yielding 16-22 pregnancies and the exposure periods were either from days 0-18 or 6-18 of pregnancy. Groups of 24-28 rabbits, yielding 20-25 pregnancies, were exposed either from days 0-21 or 7-21. Further groups of rats and rabbits were also exposed to trichloroethylene at 500 ppm for 3 weeks prior to mating, in addition to exposure during pregnancy. All mothers were killed and necropsied at the end of pregnancy and the foetuses were subjected to routine morphological examination. No clear evidence of maternal or developmental toxicity was

observed in either species. Four rabbit foetuses (from two litters) from the group exposed only during pregnancy, from days 0-21, were found with hydrocephalus. The authors believed that this finding could not "be discounted entirely as occurring by chance" because they had not previously seen this abnormality in untreated control rabbits. However, in the absence of similar findings in the other exposed groups, these abnormalities cannot realistically be attributed to trichloroethylene treatment. A limitation of this study is that it was conducted at an exposure level which did not elicit maternal toxicity.

A group of 32 mated female rats (Wistar) was exposed to trichloroethylene (distilled from Trilene®) at a concentration of 100 ppm for 4 hours/day from days 8 to 21 of pregnancy (Healy et al., 1982). A control group of 31 mated females was exposed to air under the same conditions. The females were killed on day 21 and the foetuses were examined for external, visceral and skeletal abnormalities using routine techniques. No information on maternal toxicity was presented, although at this exposure level little toxicity could be expected. The number of females with total litter loss was significantly higher in the trichloroethylene exposed group (7/32 compared with 2/31 in the control group). It is, however, difficult to interpret this finding because it is possible that the authors may have described animals showing pre-implantation loss, which would have occurred before exposure commenced, and even non-pregnant animals as having total litter loss. In the exposed group foetal weight was significantly less than the control values (by about 9%) and there was an increased incidence of minor skeletal variants, such as absent or bipartite centres of ossification. However, the results of this study have been discounted because they are inconsistent with the other inhalation studies, all of which showed no evidence of developmental toxicity at higher exposure levels.

Oral

There are three reports, emanating from the same laboratory, of studies in which the toxicity of trichloroethylene on the developing nervous system following drinking water administration to rats was investigated (Taylor et al., 1985; Noland-Gerbec et al., 1986; Isaacson and Taylor, 1989).

In the first study, the effects of maternal trichloroethylene exposure on the behaviour of the offspring was investigated *(*Taylor et al., 1985). Groups (group size unspecified) of female rats (Sprague-Dawley strain) were continuously exposed to trichloroethylene (purity unspecified) via the drinking water at concentrations of 0, 312, 625 or 1,250 mg/l for two weeks prior to mating, during pregnancy and lactation until weaning of the offspring on day 21. It was reported that the mean intake per dam of trichloroethylene during the entire exposure period was 646 mg, 1,102 mg and 1,991 mg for the 312, 625 and 1,250 mg/l groups, respectively, from which it can be estimated that the dosages were about 35, 60 and 110 mg/kg/day. Male offspring were subjected to exploratory behaviour assessments at 28, 60 and 90 days of age and their locomotor activity and feeding and drinking behaviour were monitored at 55-60 days of age. No data on maternal toxicity were presented. The exploratory activity of offspring from all the exposed groups was significantly increased at 60 days of age, although a dose-response relationship was not apparent. At 90 days the level of exploratory activity was significantly increased, in comparison with the control group, at the highest exposure level only. Also, offspring from the highest exposure group exhibited more locomotor activity, particularly during the first 3 hours of the dark cycle when the rats are normally most active.

In the second study the effect of maternal trichloroethylene exposure on glucose uptake by the offspring's brain was investigated (Noland-Gerbec et al., 1986). Groups (group size unspecified) of female rats (Sprague-Dawley strain) received trichloroethylene (purity not stated) in the drinking water at concentrations of 0 or 312 mg/l (total intake of trichloroethylene was 825 mg, estimated daily dosage 45 mg/kg/day) following the same schedule as the previous study. Two

male pups from each litter were killed at 7, 11, 16 and 21 days of age and uptake of 2-deoxyglucose in the hippocampus, cerebellum and total brain was determined by a dissection-scintillation counting method. Maternal bodyweights were not affected by treatment, although the amount of water consumed by the exposed dams was significantly less than the for the control group. At all sampling times the uptake of 2-deoxyglucose by brain tissue was significantly depressed relative to the control group (generally by about 10%), although serum glucose and 2-deoxyglucose levels for control and exposed pups were similar.

In the third study, the effect of maternal trichloroethylene exposure on myelin in the dorsal hippocampus was investigated (Isaacson and Taylor, 1989). Groups of six female rats (Sprague-Dawley strain) received trichloroethylene (purity not stated) in the drinking water at concentrations of 0, 312 or 625 mg/l (estimated daily dosages of 0, 28 and 56 mg/kg/day, based on mean water intake of 27 ml/day) following the same schedule as the previous two studies. On day 21 post partum the pups were killed and the brains were removed. Brain sections containing the hippocampus were stained using a special technique for myelin, and examined microscopically. The appearance of myelinated areas such as the internal capsule, optic tract and fornix for the exposed pups was similar to the control pups. However, an obvious decrease in the number of myelinated fibres present in the stratum lacunosum moleculare of the hippocampus was noted. The number of myelinated fibres in this region was quantitated in two or three pups from each dose group and it was found that there was a decrease of about 40% in both exposed group relative to the controls, although a dose-response was not apparent. The authors stated that it is not possible to ascertain what behavioural changes may result from such morphological changes. It is noted that a similar decrease in the numbers of myelinated fibres was seen in trichloroethylene exposed (via the water, at a concentration of 312 mg/l) weanling and young adult rats in a follow-up study; the exposed rats also exhibited enhanced performance in spatial navigational tasks (Isaacson et al., 1989), although these findings were not reproduced in a well-conducted 13-week inhalation neurotoxicity study in rats (The Dow Chemical Company, 1993) or in other neurological investigations.

The Taylor et al. (1985), Noland-Gerbec et al. (1986) and Isaacson and Taylor (1989) studies suggest that maternal exposure in the rat to trichloroethylene at dose levels in the range 30-110 mg/kg/day may induce developmental neurotoxicity, characterised by subtle changes in the behaviour (increased activity), brain metabolism (depressed 2-deoxyglucose uptake) and brain morphology (decrease in the number of myelinated fibres in one region) of the offspring. Similar morphological and behavioural changes have also been reported in trichloroethylene-exposed weanling and young adult rats in studies conducted by the same group, but these findings have not been reproduced elsewhere. In assessing the concerns that these results may raise for human health, a first consideration is the quality of the studies. The major limitation is that group sizes were small or unspecified, such that it is difficult to draw definitive conclusions with respect to either qualitative or quantitative aspects on the basis of these studies. A second consideration is the biological significance of the observed changes; at present it is not known for certain if these changes have any biological significance, although any changes in the brain morphology should be viewed with concern. Overall, it is not possible to draw clear conclusions about the relevance of these findings to human health without corroborative evidence from elsewhere.

The developmental toxicity of trichloroethylene has been studied in mice (Cosby and Dukelow, 1992). Groups of at least 10 females (C57BL/6 x DBA/2 (B6D2F1) strain) were administered trichloroethylene (spectrophotometric-grade) as a solution in corn oil by gavage from either days 1 - 5, 6 - 10 or 11 - 15 of pregnancy at dose levels of 0, 24 and 240 mg/kg/day. The mothers were allowed to litter and rear their offspring until weaning. For the offspring, litter size, weight,

crown-rump length, gross external abnormalities and survival were recorded. Also, for some pups the gonad weights were recorded. The offspring were killed either at weaning or at 6 weeks of age. According to the authors, no evidence of maternal or developmental toxicity was seen, but very few data were presented so it was not possible to verify the authors' conclusion. The weight that can be given to this conclusion is considerably reduced because of certain limitations of the study, notably the small group size, brief reporting style and absence of detailed morphological examination of the offspring.

Summary of developmental toxicity

The developmental toxicity of inhaled trichloroethylene at non-maternally toxic levels (up to 1,800 ppm) has been investigated in rats, mice and rabbits in conventional studies. No evidence of developmental toxicity was reported. In contrast, the results of a series of non-standard oral studies in rats raised some concerns about the potential for trichloroethylene to induce developmental neurotoxicity at dose levels in the range of 30-110 mg/kg/day. However, these studies were of limited scope and were considered not to provide sufficient basis on which to draw clear conclusions about the hazardous properties of trichloroethylene.

4.1.2.9.2 Human reproduction

Fertility and reproductive performance

The possible effects of trichloroethylene on male or female fertility have not been investigated in humans.

HSE (1982) cites several isolated briefly-reported studies in which complaints of reduced libido or decreased potency among trichloroethylene exposed male workers (Bardodej and Vyskocil, 1956; El Ghawabi et al., 1973) and an increased incidence of menstrual disorders among women who may have been exposed to relatively high concentrations of trichloroethylene (Bardodej and Vyskocil, 1956; Zielinski, 1973) were described. However, the reports did not give sufficient details to allow the significance of these findings to be assessed.

Developmental toxicity

The possible association between trichloroethylene exposure and certain adverse pregnancy outcomes have been investigated in several studies.

In 1973 it was observed that a relatively high proportion of patients with congenital heart disease in Tucson Valley, Arizona, came from one particular area of the city. Following the discovery in 1981 that the drinking water supplied to this area was contaminated with trichloroethylene, and to a lesser extent with dichloroethylene and chromium, a formal study was conducted to test the hypothesis that the incidence of congenital heart disease was higher among those whose parents had been in contact with the contaminated water (Goldberg et al., 1990). Trichloroethylene levels in the water supply were measured in 1981 and ranged from 6 to 239 ppm; dichloroethylene levels were about 5 to 10% of the trichloroethylene levels; chromium levels, which had been measured from the 1950s, were described as approaching but not exceeding the local action levels. It was thought that contaminated water had been supplied to this area from the 1950s; the contaminated wells were closed in 1981. About 9% of the Tucson valley population lived in the contaminated water area.

From hospital records 707 cases with a verified diagnosis of congenital heart disease, with one or both parents living in the Tucson area for at least one month before and during the first trimester of the affected pregnancy were identified among children who were born in the Tucson area between 1969 and 1987. The families were contacted and information on parental residence and work location, age, occupation, educational level, ethnicity and medical history (rubella infection disqualified participation) was obtained by interview. A further 218 cases which satisfied the inclusion criteria with respect to heart disease were identified, but the investigators were unable to contact the families. Of the 707 cases, 246 (35%) had parental contact (either through work or residential location) with the contaminated water area, compared with an expected percentage of about 10%, a difference which was statistically significant. The prevalence of congenital heart disease among the offspring of mothers resident in the contaminated water area during the period of active contamination was 0.68%, compared with a prevalence of 0.26% for a randomly selected control group of mothers resident in non-contaminated areas during this period. Further analyses were conducted to determine the influence of paternal contact with contaminated water through work or residential contacts or maternal contact through work but these were found not to be significant risk factors.

However, the authors stated that the overall prevalence of congenital cardiac defects (of the type that qualified for inclusion in the study) in the Tucson valley for the study period, including those born to mothers who lived outside this area during the first trimester, was 0.7% of live births. This suggests that the prevalence of cardiac disease in the contaminated area was similar to the background prevalence. For this reason, and because it was not possible to characterise trichloroethylene dose levels in the exposed group, it is considered that this study presents no convincing evidence of an association between trichloroethylene exposure and congenital cardiac abnormalities.

The effects of paternal and maternal occupational exposure to trichloroethylene and other organic solvents on pregnancy outcome were investigated in two Finnish case-control studies (Taskinen et al., 1989; Lindbohm et al., 1990). Both studies were nested in cohorts of men and women who had participated in a biological monitoring program for organic solvent exposure between 1965 and 1983. Information on the pregnancy outcomes of wives was obtained from national medical registries. In neither study were any quantitative exposure data available.

In the first study, the association between paternal exposure and spontaneous abortions and congenital malformations was investigated (Taskinen et al., 1989). Cases were defined as wives with a spontaneous abortion or with a congenitally malformed child. Control pregnancies were matched for maternal age; three were selected for each abortion and five for each malformation. Paternal exposure for the period of 80 days prior to conception was assessed from questionnaire replies and, where available, biological monitoring data. Additional information was obtained by questionnaire on both paternal and maternal health and lifestyle factors and maternal occupational exposure. A final total of 120 cases of abortion and 25 cases of malformation was included in the study. It was found that paternal trichloroethylene exposure was not a risk factor for spontaneous abortion, since the proportion of cases with paternal trichloroethylene exposure was similar to that of controls (odds ratio 1.0, 95% confidence interval 0.6-2.0). Also, organic solvent exposure did not appear to be a risk factor for congenital malformations, but the number of cases of malformations was to small to allow an analysis with respect to trichloroethylene exposure.

In the second study, the association between maternal occupational solvent exposure and spontaneous abortion was investigated (Lindbohm et al., 1990). Women with abortions were defined as cases. Three control pregnancies, matched for age, were selected for each case. Trichloroethylene exposure was assessed from questionnaire response and, where available,

biological monitoring data. Additional information was obtained by questionnaire on health and lifestyle factors. A final total of 73 cases were included. The odds ratio for trichloroethylene exposure was 0.6 (95% confidence interval 0.2 - 2.3), suggesting that trichloroethylene is not a risk factor for spontaneous abortion.

As part of a mortality study, also conducted in Finland, information on the incidence of congenital malformations among the offspring of 969 women identified as having been occupationally exposed to trichloroethylene at some time between 1963 and 1976 (Tola et al., 1980, cited in HSE, 1982, see human cancer/epidemiology section for further details). The incidence of malformations was obtained from a national register, to which reporting is compulsory; this registration scheme is estimated to have a reporting/detection failure rate of 30% (Taskinen et al., 1989). No information on exposure levels which relates specifically to women is presented, although for the total cohort, which included 1,148 men, the highest recorded urinary trichloroacetic acid level was below 100 mg/l (suggesting that airborne exposure levels were generally less than 40 ppm). No malformed offspring were reported as having been born to any of the exposed women. Although the exact number of pregnancies occurring in the study cohort was no known, it was estimated, based on national figures for fertility rates and incidence of congenital malformations, that about three malformed offspring would have been expected in this population. This study indicates that occupational trichloroethylene exposure is not a risk factor for congenital malformations among Finnish women.

These three Finnish studies provide evidence that trichloroethylene exposure at the levels experienced in that country's industry are not associated with increased risks of spontaneous abortion or congenital malformation. However, a major limitation of these studies is the poor characterisation of exposure levels, which limits the contribution these studies can make to a broader assessment of the effects of trichloroethylene exposure on pregnancy outcome in humans.

To summarise the human data, the potential effects of trichloroethylene exposure on reproduction in humans has not been extensively investigated. Several studies have failed to demonstrate an association between occupational exposure and abortion or congenital malformations, but these studies were of limited value because exposure levels could not be quantified. Overall, it is not possible to draw any firm conclusions about the reproductive toxicity of trichloroethylene on the basis of the human data.

4.1.2.9.3 Summary of toxicity for reproduction

In animals, the effects of long-term oral administration on fertility and reproductive performance have been extensively investigated in rats and mice. Trichloroethylene was shown to have some influences on reproduction and only at exposure levels that produce general toxicity. The observed effects included reduced sperm motility and reductions in neonatal bodyweight and survival in mice at high-dose levels and, in rats, disrupted copulatory behaviour and reduced pup survival at high-dose levels, and reductions in the litter size and number of litters born to continuously bred animals. NOAELs for reproductive effects were 350 mg/kg/day in mice and 75 mg/kg/day in rats.

Two studies in mice have suggested that short-term repeated inhalation exposure can influence sperm morphology; a NOAEL for this effect was 200 ppm. However, effects on sperm morphology were not seen in rats, nor in large-scale studies in which mice received long-term exposure to high levels of trichloroethylene by the oral route. The developmental toxicity of

inhaled trichloroethylene at non-maternally toxic levels (up to 1,800 ppm) has been investigated in rats, mice and rabbits in conventional studies. No evidence of developmental toxicity was reported. In contrast, the results of a series of non-standard oral studies in rats raised some concerns about the potential for trichloroethylene to induce developmental neurotoxicity at dose levels in the range of 30-110 mg/kg/day. However, these studies were of limited scope and were considered not to provide sufficient basis on which to draw clear conclusions about the hazardous properties of trichloroethylene. To be able to draw clear conclusions regarding developmental neurotoxicity, further testing according to the draft OECD TG 426 Developmental Neurotoxicity guideline would be required.

It is not possible to draw any firm conclusions about the reproductive toxicity of trichloroethylene on the basis of the available human data.

4.1.3 Risk Characterisation

4.1.3.1 General aspects

Studies in experimental animals and humans have shown that trichloroethylene is rapidly and extensively absorbed by all routes of exposure. For the purposes of risk assessment 100% absorption by all routes of exposure will be assumed.

Once absorbed it readily distributes to all compartments within the body. Although trichloroethylene preferentially partitions into fat rich tissues, there is no evidence of prolonged retention at these sites. Trichloroethylene is predominantly cleared from the body by metabolism, accounting for 50 to 99% of the absorbed dose. Studies in humans and a variety of experimental animal species suggest that the metabolic pathways are common to all species although there are differences between species and strains in the saturability of trichloroethylene metabolism. No evidence of saturation for any metabolic pathway has been found in humans although the exposure levels were generally lower than those used in animal studies and thus saturable concentrations may not have been achieved.

The main toxic effect associated with acute inhalation exposure is CNS depression. Exposure to very high concentrations causes narcosis; extensive experience in the use of trichloroethylene as an anaesthetic at concentrations of 5,000 to 10,000 ppm has demonstrated that recovery from narcosis is usually complete. Studies in human volunteers have shown that the NOAEL for CNS depression is about 300 ppm, for exposures of up to eight hours. CNS depression can also occur in humans following oral ingestion of doses of about 450 mg/kg and above. The acute animal data are generally consistent with the human observations. In the rat, an inhalation 4-hour LC_{50} value of 12,000 ppm and oral LD_{50} values of 5,400 to 7,200 mg/kg are reported. For the dermal route there are no human data and little animal data; in rabbits the dermal LD_{50} exceeds 2,000 mg/kg. In mice, some subtle effects in the lung (vacuolation of a few Clara cells) were seen at inhalation exposure levels as low as 20 ppm; however, there is evidence that this effect is related to the way in which the mouse metabolises trichloroethylene, so this effect is thought unlikely to be of relevance to humans.

Human experience and limited animal experimental data indicate that liquid trichloroethylene is a skin and eye irritant. The skin or respiratory sensitising potential has not been investigated in animal models. In humans there are very occasional reports of reactions which may be due to trichloroethylene-related skin sensitisation, but if so, these are likely to be rare idiosyncratic reactions given the large number of people that have been exposed to trichloroethylene. Trichloroethylene is considered not to have significant skin sensitising potential. Also, the absence of reports of respiratory problems in trichloroethylene exposed humans, given the large number of workers exposed to trichloroethylene via the inhalation route, indicates that it is reasonable to assume that it is not a respiratory sensitiser.

The repeated dose toxicity of trichloroethylene in humans has not been well characterised, although there is an extensive database. In limited volunteer studies no evidence of toxicity was seen following repeated short-term exposure of up to 200 ppm for up to 7.5 hours/day. There are numerous reports of health surveys being carried out on workers occupationally exposed to trichloroethylene, where exposure is primarily by inhalation, although some dermal exposure is also likely. Unfortunately, the value of these studies is severely limited by the lack of any detailed information on the atmospheric trichloroethylene levels, the possibility of exposure to other chemicals including alcohol and other potential confounding factors. Furthermore, in many studies, no control group was used. Consequently, it is difficult to assess either the qualitative or

quantitative relationship that any observed health effects or symptoms may have with trichloroethylene exposure. Most studies report the presence of subjective symptoms of CNS disturbance in exposed workers. Consistently reported symptoms include fatigue, vertigo, dizziness, headaches, memory loss and impaired ability to concentrate. Also, there are a number of reports of skin and eye irritation. The consistency of the reports lends support to the view that these symptoms were related to trichloroethylene exposure and therefore functional CNS disturbance is regarded as a key endpoint in humans, although the available data do not allow conclusive judgements to be made regarding causal or dose-response relationships. Notwithstanding the dose-response uncertainties, the overall view reached by the majority of Member States in discussing this endpoint is that there appears to be an absence of CNS effects associated with exposure levels of around 50 ppm and taking a cautious approach, this value was identified as a NOAEL for functional CNS disturbance. However, it is noted that in animal studies, a clear NOAEL for neurotoxicity of 200 ppm has been identified for long-term inhalation exposure to trichloroethylene. In view of this, and given the uncertainties surrounding the identification of a reliable NOAEL for humans from the available data, it is possible that NOAEL for neurotoxicity in humans may be higher than 50 ppm. Intolerance to alcohol, presenting as a transient redness affecting mainly the face and neck (generally known as "trichloroethylene or degreasers' flush") has also been frequently observed in exposed workers. Identical effects have been seen in single and repeated dose volunteer studies, confirming that this effect is caused by trichloroethylene. Some studies included special investigations for liver, cardiac or neurological toxicity, but the data were too limited to allow any definitive conclusions to be drawn. There are occasional case reports describing patients who had been exposed to trichloroethylene at work with conditions such as Stevens-Johnson syndrome or scleroderma. It is possible that these may represent rare idiosyncratic reactions to trichloroethylene, but with the available information it is not possible to draw any conclusions about causality.

In animals, the main toxic effects which have been observed following repeated inhalation exposure to trichloroethylene are on the liver, kidney, CNS, pulmonary system and hearing. Liver and kidney toxicity have also been reported following oral administration. Overall, in animals, kidney toxicity appears to be the most sensitive endpoint for both long-term repeated inhalation and oral exposure. NOAELs of 100 ppm and 50 mg/kg/day were identified in rodents for inhalation and oral exposure, respectively. Comparing the human and animal data, there are no reports of trichloroethylene-related kidney toxicity in the human studies, but this endpoint has not been properly investigated in humans. Therefore, because of the paucity of human data for this endpoint, the animal data must be considered in the risk characterisation, and a NOAEL of 100 ppm is identified for kidney toxicity.

The genotoxicity of trichloroethylene has been extensively investigated in experimental test systems. Trichloroethylene tested positive in a bacterial (Ames) test and a mouse lymphoma gene mutation assay, demonstrating that trichloroethylene is an *in vitro* mutagen. There is contradictory evidence of *in vivo* genotoxic activity and consequently there have been uncertainties about whether trichloroethylene should be classified for potential mutagenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity. The majority of the members of this group recommended, based on a weight of evidence assessment approach, that the positive evidence for DNA interaction justified classification as a category 3 mutagen. This indicates that, in the opinion of the majority of Specialised Experts, the substance causes concerns for humans owing to possible mutagenic effects. In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly.

The carcinogenicity of trichloroethylene has been investigated in a number of long-term animal studies, using the oral and inhalation routes, and involving hamsters and a variety of strains of rat and mouse. These studies provide clear evidence that trichloroethylene is carcinogenic in rats and mice. In the mouse, trichloroethylene induced hepatocellular tumours by either the inhalation or oral routes and lung tumours by the inhalation route. The hepatocellular tumours were observed at high oral doses levels of 1,000 mg/kg/day and above (lower exposure levels were not investigated) and by inhalation, at 600 ppm, but not at 300 ppm. The lung tumours occurred at exposure levels as low as 150 ppm in one study but not at 100 ppm in another. In the rat, trichloroethylene induced renal tubular adenomas, albeit at a low incidence, in association with other pathological changes. Kidney tumours were induced at an exposure level of 600 ppm but not at 300 ppm inhalation exposure; by oral exposures kidney tumours were seen at 500 mg/kg/day and above in some studies but not at 250 mg/kg/day in another. Since there are uncertainties regarding the role of genotoxicity in trichloroethylene induced carcinogenicity, other mechanisms of tumour formation, and their possible relevance to humans, should be considered. With regard to the liver tumours seen in mice, there is evidence that these are associated with the phenomenon of peroxisome proliferation, produced by the metabolite trichloroacetic acid. The extent of formation of this metabolite is much greater in mice than in rats, a species which does not develop liver tumours following trichloroethylene exposure, and in humans. Furthermore, humans are much less sensitive to the induction of peroxisome proliferation, compared with rodents. Accordingly, it is considered that the induction of liver tumours in mice is unlikely to be of relevance to human health. Turning to the lung tumours seen in mice, there is evidence to suggest that these may be associated with the accumulation of the metabolite chloral hydrate in Clara cells and that the accumulation of this metabolite is unlikely to occur in humans. There is also uncertainty about the mechanism by which the kidney tumours arise in rats. There is no evidence of hyaline droplet nephropathy, a mechanism associated with male rat-specific kidney tumour formation. One possibility is that the kidney tumours are related to mutagenic activity or repeated cycles of cytotoxicity and proliferation, perhaps related to β-lyase activation of the mutagenic trichloroethylene metabolite DCVC or to excess formic acid excretion, but little is known about the toxicity of trichloroethylene to the kidney in humans so it is difficult to judge the relevance of these tumours to human health.

The carcinogenicity of trichloroethylene has also been investigated in occupationally exposed populations in a number of studies. The majority of these did not show any evidence that trichloroethylene exposure is associated with an increased incidence of cancer. Individually, each of the negative studies had certain limitations although several, by nature of their design, had substantial power to detect an effect, and when taken together provide significant evidence that trichloroethylene is not carcinogenic in humans under the exposure conditions experienced by the groups studied. However, the occupational studies did not provide complete reassurance of the absence of trichloroethylene-related carcinogenicity in humans. This was because limited evidence of an increased risk of cancer among trichloroethylene exposed workers was reported in one well-conducted cohort study, although definitive conclusions about causality could not be made in view of the limited corroboration with the results of the other studies. Furthermore, in two other studies an increased risk of kidney cancer was reported in a group of trichloroethylene workers; although it was not possible to draw any conclusion from these studies because of methodological weaknesses, this association adds to the concerns about the carcinogenic potential of trichloroethylene. Overall, given the observation of kidney tumours in animal studies and absence of convincing evidence that these arose by mechanisms not applicable to humans, together with the uncertainties regarding the human data, it is considered that there remains some concern for potential carcinogenicity in humans.

There have been uncertainties about whether trichloroethylene should be classified for potential carcinogenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity. The majority of the members of this group concluded that classification as a category 2 carcinogen is warranted, based on evidence of carcinogenicity in one animal species, namely tumours in the rat kidney, supported by epidemiological data showing an association between exposure and kidney tumours and non-Hodgkin's lymphoma in humans. This indicates that, in the opinion of the majority of Specialised Experts, trichloroethylene should be regarded as if the substance is carcinogenic to humans. In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly.

Accordingly, carcinogenicity is regarded as a key health effect in the risk characterisation for human health. Because of uncertainties about the mode of action it is not possible to draw any conclusions with regard to the presence of an identifiable threshold level of exposure below which there is no increased risk.

It is not possible to draw any firm conclusions about the reproductive toxicity of trichloroethylene on the basis of the available human data; accordingly, the risk characterisation for reproductive effects must be based on animal data. Effects of long-term oral administration on fertility and reproductive performance have been extensively investigated in rats and mice. Trichloroethylene was shown to have some influences on reproduction and only at exposure levels that produce general toxicity in adult animals. The observed effects included reduced sperm motility and reductions in neonatal bodyweight and survival in mice at high-dose levels and, in rats, disrupted copulatory behaviour and reduced pup survival at high-dose levels, and reductions in the litter size and number of litters born to continuously bred animals. NOAELs for reproductive effects with oral dosing were 350 mg/kg/day in mice and 75 mg/kg/day in rats. Two studies in mice have suggested that short-term repeated inhalation exposure can influence sperm morphology; a NOAEL for this effect was 200 ppm. However, effects on sperm morphology were not seen in rats, nor in large-scale studies in which mice received long-term exposure to high levels of trichloroethylene by the oral route. The reproductive NOAELs have not been considered in the risk characterisation because these are greater than the NOAELs for repeated dose toxicity. The developmental toxicity of inhaled trichloroethylene at non-maternally toxic levels (up to 1,800 ppm) has been investigated in rats, mice and rabbits in conventional studies. No evidence of developmental toxicity was reported. In contrast, the results of a series of non-standard oral studies in rats raise concerns about the potential for trichloroethylene to induce developmental neurotoxicity at dose levels in the range of 30-110 mg/kg/day. However, these studies were of limited scope and were considered not to provide sufficient basis on which to draw clear conclusions about the hazardous properties of trichloroethylene. To be able to draw clear conclusions regarding developmental neurotoxicity, further testing according to the draft OECD TG 426 would be required. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen, the results of such testing are unlikely to influence the outcome of the risk assessment. This is because the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

Overall, the hazardous properties of trichloroethylene have been evaluated to the extent that the minimum data requirements according to Article 9(2) of Regulation 793/93 have been met. The key health effects of acute toxicity, skin and eye irritation, repeated dose toxicity, mutagenicity and carcinogenicity have been identified. For acute toxicity, a NOAEL of 300 ppm has been identified in humans for CNS depression. No quantitative data are available for skin and eye

irritation. For repeated dose toxicity, a precautionary NOAEL of 50 ppm for functional CNS disturbance in humans has been identified. Additionally, a repeated dose NOAEL of 100 ppm for kidney toxicity is derived from animal studies. For mutagenicity and carcinogenicity it is assumed for risk characterisation purposes that there is no identifiable threshold exposure level.

There are no concerns for sensitisation.

4.1.3.2 Workers

4.1.3.2.1 Manufacture and recycling

It is estimated that between 400 and 700 workers in the EU are exposed to trichloroethylene during manufacture. The main route of exposure is via inhalation. Personal exposure data are available from a UK manufacturing plant, for workers involved in production and packing. The majority of 8-hour time weighted average exposures were below 10 ppm. Very occasionally, personal-sampling atmospheric 8-hour TWAs above 100 ppm and as high as 590 ppm were reported, possibly due to spillages, but respiratory protective equipment was worn when such conditions were anticipated. In two Italian plants, static sampling has indicated exposures to atmospheric concentrations of between 0.1 and 9.1 ppm during manufacture.

For manufacture, dermal exposure was predicted using EASE to be in the range 0 to 0.1 mg/cm^2/day, although on most days no significant contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range. However, taking a worst-case approach and assuming that exposure up to 0.1 mg/cm^2/day occurs, this could lead to a total estimated exposure of about 80 mg/worker/day, assuming that the area of two hands (820 cm^2) is exposed. Toxicokinetic data show that trichloroethylene is rapidly and extensively absorbed by this route so, adopting a cautious approach, it is assumed that absorption will be 100% of the applied dose. Thus, a 70 kg worker could potentially receive a body burden of 1 mg/kg/day from dermal exposure.

A relatively small number of persons are exposed to trichloroethylene during recycling. The main route of exposure is via inhalation. Data from one UK plant gave 8-hour TWA values of <1 to 9 ppm (mean 2.7 ppm).

For recycling, dermal exposure was predicted using EASE to be in the range 0 to 0.1 mg/cm^2/day, although on most days no significant contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range. However, again taking a worst-case approach and assuming that exposure up to 0.1 mg/cm^2/day occurs, this could lead to a total estimated exposure of 80 mg/worker/day, assuming that the area of two hands (820 cm^2) is exposed. Thus, a 70 kg worker could potentially receive a body burden of about 1 mg/kg/day from dermal exposure, assuming 100% dermal absorption.

To assess the potential total body burden for workers exposed during manufacture and recycling, the dermal estimates must be combined with that for inhalation exposure. For exposure to an atmospheric concentration of up to 10 ppm (55 mg/m^3), assuming that 10 m^3 of air is inhaled in a working day and 100% of the inhaled dose is absorbed, a 70 kg worker could receive a body burden of up to 8 mg/kg/day. A total body burden from inhalation and dermal exposure combined could therefore be up to about 9 mg/kg/day.

For comparison with the body burden arising from workplace exposure, the quantitative rat toxicity data have been converted to an estimated body burden using the assumptions of 100% absorption by the inhalation route, 350 g bodyweight and a breathing rate of 6 l/hr. The quantitative human data have been converted to an estimated body burden using the assumptions indicated above.

The extent to which exposure during breaches of closed systems can be controlled during manufacture and recycling, will depend on the technology adopted. For example, tanker filling could typically range from the splash filling to the use of dry-break coupling systems, the latter providing a greater degree of control. Similarly for product sampling or maintenance there are a variety of different ways of carrying out the activity, each with a different exposure profile. The extent to which a manufacturer or a company involved in the recycling of trichloroethylene can be considered to have reduced exposure as far as is reasonably practicable will depend on the extent to which high standards of control have been adopted. For carcinogens, best practice would be considered to include, as far as is reasonably practicable, the use of, for example, dry-break coupling systems for tanker loading and off loading, or the use of closed or ventilated sampling points.

Comparison of exposure and effects for manufacture and recycling

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm (1,640 mg/m^3), equivalent to a body burden of 235 mg/kg/day. The highest estimated body burden arising from combined inhalation and dermal exposure during manufacture and recycling is 9 mg/kg/day. This is a factor of 26 below the human NOAEL. A MOS of this magnitude provides reassurance that adverse health effects will not occur, even allowing for variation between individuals in their sensitivity to this endpoint. Therefore **conclusion (ii)** is reached.

Irritation

The skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because exposure is negligible, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye, which could occur accidentally, then local damage is possible. Overall, however, **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the human NOAEL of 50 ppm equates to a body burden of 38 mg/kg/day. The highest body burden from combined inhalation and dermal exposure during manufacture and recycling is 9 mg/kg/day. Thus, the MOS is 4. Although relatively low, this MOS is considered acceptable because the NOAEL is a precautionary estimate, based on human data, and account should be taken of the much higher animal NOAEL of 200 ppm for this endpoint, which suggests that the human NOAEL could be higher. Thus, **conclusion (ii)** is reached for this endpoint.

For kidney toxicity, the NOAEL of 100 ppm for the rat equates to a body burden of about 65 mg/kg/day. The highest body burden for manufacture and recycling is 9 mg/kg/day. Thus, the MOS is 7; an MOS of this magnitude raises concerns for human health, as it may not be sufficient to allow for differences in toxicokinetics and toxicodynamics between and within species. Thus, **conclusion (iii)** is reached for this endpoint.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. Although high standards of control are available in these industry sectors, representing best practice for a substance with these properties, there is no evidence that these standards are currently applied consistently across all EU industry. Thus, there is no evidence that the appropriate equipment is in place in all EU workplaces and that it is used and maintained in the correct manner. Therefore it is considered that risk reduction measures are required, and **conclusion (iii)** applies.

Table 4.14 Summary of risk characterisation for workers during manufacture and recycling

Key health effect	Human exposure	Quantitative toxicity data	MOS	Conclusion
Acute toxicity	9 mg/kg/day, 8 h (body burden)	NOAEL 235 mg/kg/day in humans	26	ii
Irritation (skin and eye)	Negligible for skin and eye	No quantitative data	-	ii
Repeat dose toxicity: functional CNS disturbance	9 mg/kg/day, 8 h (body burden)	NOAEL 38 mg/kg/day (body burden) in humans	4	ii
Repeat dose toxicity: kidney toxicity	9 mg/kg/day, 8 h (body burden)	NOAEL 65 mg/kg/day in animals	7	**iii**
Mutagenicity	9 mg/kg/day, 8 h (body burden)	Non-threshold assumed	-	**iii**
Carcinogenicity	9 mg/kg/day, 8 h (body burden)	Non-threshold assumed	-	**iii**

4.1.3.2.2 Metal cleaning

The numbers of workers exposed to trichloroethylene in degreasing units in the EU could be in excess of 60,000. Workers are likely to be exposed by both the inhalation and dermal routes.

UK data from several sources indicate that workplace inhalation exposures (8-hour TWA) can be controlled to below 20 ppm for a well maintained and correctly operated degreasing bath. Occupational exposures are likely to be lower during the use of modern enclosed degreasing baths. However, exposures are often higher than 20 ppm 8-hour TWA during the use of open top degreasing baths. The 90/95 percentile approximates to 50 ppm 8-hour TWA for the data received. For short-term exposure, the results of measurements taken by HSE and industry indicate that exposures during loading/unloading of the degreasing bath are on many occasions above the current UK short-term occupational exposure limit of 150 ppm and may be as high as 500 ppm. These short-term exposures may result in high shift exposures. These high short-term exposures were found where companies had failed to adequately maintain the degreasing bath or where working practices were poor. During the cleaning of degreasing baths where the operator enters the bath short-term exposures in excess of 5,000 ppm have been estimated, although in these situations control of exposure is achieved by the provision of suitable breathing apparatus. Also, improvements in bath design are removing the need for operators to enter the baths.

For dermal exposure, no data are available from industry. Modelled data (using EASE) indicated that exposure could be as high as 1 mg/cm^2/day, assuming regular contact and minimal handling precautions. This could lead to a total estimated exposure of 2,000 mg/worker/day, assuming an

area of 2,000 cm^2 of skin on the hands and forearms is exposed. Toxicokinetic data show that trichloroethylene is rapidly and extensively absorbed by this route so, adopting a cautious approach, it is assumed that absorption will be 100% of the applied dose. Thus, a 70 kg worker could potentially receive a body burden of 30 mg/kg/day from dermal exposure.

To assess the potential total body burden for metal cleaning workers, the dermal estimates must be combined with that for inhalation exposure. Assuming exposure to an atmospheric concentration of 50 ppm (265 mg/m^3) during the use of open top degreasing baths, 10 m^3 of air is inhaled in a working day and 100% of the inhaled dose is absorbed, a 70 kg worker will receive a body burden of 38 mg/kg/day. A total body burden from inhalation and dermal exposure combined could therefore be as high as 68 mg/kg/day.

For comparison with the body burden arising from workplace exposure, the quantitative rat toxicity data have been converted to an estimated body burden using the assumptions of 100% absorption by the inhalation route, 350 g bodyweight and a breathing rate of 6 l/hr. The quantitative human data have been converted to an estimated body burden using the assumptions indicated above.

Open top hot vapour degreasing equipment is still in use in some EU member states. The control of occupational exposure during the use of these baths is dependent on the equipment being well maintained. In addition, the bath needs to be correctly used, for example, hoist speeds should not be too fast and components should be correctly loaded. Therefore where the baths are not well maintained and correctly used, exposures will be higher. These baths are open to the workplace, therefore any failure results in an immediate increase in the amount of vapour emitted from the bath. In conclusion, although occupational exposure can be controlled to less than 20 ppm 8-hour TWA using open top baths, it is far more likely for exposure to be higher. Modern enclosed baths have far better solvent recovery. The hot vapour cleaning zone is not exposed to atmosphere. Although maintenance is again important there is far less opportunity for the operator to increase his / her own exposure, since the operator has no direct contact with the solvent, other than any residual solvent remaining on the components. These modern enclosed baths can therefore be regarded as representing best practice for hot vapour degreasing.

Comparison of exposure and effects for metal cleaning

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm (1,640 $mg.m^{-3}$), equivalent to a body burden of 235 mg/kg/day. The estimated body burden from inhalation and dermal exposures combined during metal cleaning operations is 70 mg/kg/day, which is a factor of about 3 below the human NOAEL. Additionally, short-term peak exposures of 500 ppm are possible; such exposures are greater than the human NOAEL. Accordingly, there are concerns for human health because of these short-term high peak exposures and **conclusion (iii)** is reached.

Irritation

The skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because exposure is low, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye, which could occur accidentally, then local damage is possible. Overall, however, **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the human NOAEL of 50 ppm equates to a body burden of 38 mg/kg/day. The body burden from combined inhalation and dermal exposure during metal cleaning is 68 mg/kg/day. Thus, the MOS is less than 1, which raises concerns for human health and **conclusion (iii)** is reached.

For kidney toxicity, the NOAEL of 100 ppm for the rat equates to a body burden of about 65 mg/kg/day. The comparison with the body burden for metal cleaning of 68 mg/kg gives an MOS of about 1, which raises concerns for human health and thus **conclusion (iii)** is reached.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. Although high standards of control are available in this industry sector, representing best practice for a substance with these properties, there is no evidence that these standards are currently applied consistently across all EU industry. Thus, there is no evidence that the appropriate equipment is in place in all EU workplaces and that it is used and maintained in the correct manner. Therefore it is considered that risk reduction measures are required, and **conclusion (iii)** applies.

Table 4.15 Summary of risk characterisation for workers during metal cleaning

Key health effect	Human exposure	Quantitative toxicity data (body burden)	MOS	Conclusion
Acute toxicity	68 mg/kg/day, 8h (body burden) 500 ppm short-term peak	NOAEL 235 mg/kg/day (body burden) in humans NOAEL 300 ppm in humans	3 0.6	**iii**
Irritation (skin and eye)	Low, provided good occupational hygiene practices are in operation	No quantitative data	-	ii
Repeat dose toxicity: functional CNS disturbance	68 mg/kg/day (body burden)	NOAEL 38 mg/kg/day (body burden) in humans	0.5	**iii**
Repeat dose toxicity: kidney	68 mg/kg/day (body burden)	NOAEL 65 mg/kg/day (body burden) in animals	1	**iii**
Mutagenicity	68 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**
Carcinogenicity	68 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**

4.1.3.2.3 Adhesive manufacture

A relatively small number of workers are exposed to trichloroethylene during the manufacture of trichloroethylene-based adhesives. Exposures for the manufacture of adhesives using open mixing vessels were predicted with EASE, although many plants may use closed systems. Where LEV is used on open mixing vessels 10 to 20 ppm 8-hour TWA was predicted and where LEV is not used 100 to 140 ppm 8-hour TWA was predicted. Exposures during charging / discharging where mixing is in closed vessels are likely to be lower than these values. It is also worth noting that the above predictions assume that the operator is only exposed to trichloroethylene vapour and spends the full shift near the mixing vessel. Since only a proportion of the blend will be trichloroethylene and the operator is unlikely to be near the vessel for the full shift, the above

exposures are likely to be overestimates. The use of LEV can be considered to be the minimum standard of control for this use, therefore for most plants exposures of up to 140 ppm 8-hour TWA can be considered to be an over estimate. Higher standard of control will be achieved where mixing is carried out in a closed vessel, with the potential for exposure only occurring during charging and discharging. Such closed systems can be regarded as best practice for the industry.

Dermal exposure is also possible. Using the EASE model, exposure as high as 1 mg/cm^2 is predicted; this value has been adopted as the worst reasonable case exposure via the dermal route. This could lead to a total estimated exposure of 2,000 mg/worker/day, assuming an area of 2,000 cm^2 of skin on the hands and forearms is exposed. Toxicokinetic data show that trichloroethylene is rapidly and extensively absorbed by this route so, adopting a cautious approach, it is assumed that absorption will be 100% of the applied dose. Thus, a 70 kg worker could potentially receive a body burden of 30 mg/kg/day from dermal exposure.

To assess the potential total body burden for adhesive manufacturing workers, the dermal estimates must be combined with that for inhalation exposure. Assuming exposure to an atmospheric concentration of 20 ppm when LEV is used, 10 m^3 of air is inhaled in a working day and 100% of the inhaled dose is absorbed, a 70 kg worker will receive a body burden of 15 mg/kg/day. A total body burden from inhalation and dermal exposure combined could therefore be as high as 45 mg/kg/day. If LEV is not used, an atmospheric concentration of 140 ppm will result in a potential body burden of 105 mg/kg/day from inhalation exposure and 135 mg/kg/day for combined inhalation and dermal exposure.

For the comparison of exposure and effects, the quantitative toxicity data have been converted to an estimated body burden using the assumptions given above for metal cleaning.

Comparison of exposure and effects for adhesive manufacture, assuming LEV is used

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm, equivalent to a body burden of 235 mg/kg/day. The estimated body burden from inhalation and dermal exposures combined during adhesive manufacture is 45 mg/kg/day, which is a factor of 5 below the human NOAEL. A MOS of this magnitude, taking account of the fact that the NOAEL is based on human data, does not raise concerns for human health, even allowing for variation between individuals in their sensitivity to this endpoint. Therefore **conclusion (ii)** is reached.

Irritation

The skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because exposure is low, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye, which could occur accidentally, then local damage is possible. Overall, however, **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the human NOAEL of 50 ppm equates to a body burden of 38 mg/kg/day. The body burden from combined inhalation and dermal exposure during adhesive manufacture is 45 mg/kg/day. Thus, the MOS is less than 1, which raises concerns for human health and thus **conclusion (iii)** is reached.

For kidney toxicity, the NOAEL of 100 ppm for the rat equates to a body burden of 65 mg/kg/day. The comparison with the body burden of 45 mg/kg for adhesive manufacture gives an MOS of about 1.5, which raises concerns for human health, as it may not be sufficient to allow for differences in toxicokinetics and toxicodynamics between and within species. Thus **conclusion (iii)** is reached.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. Although high standards of control are available in this industry sector, representing best practice for a substance with these properties, there is no evidence that these standards are currently applied consistently across all EU industry. Thus, there is no evidence that the appropriate equipment is in place in all EU workplaces and that it is used and maintained in the correct manner. Therefore it is considered that risk reduction measures are required, and **conclusion (iii)** applies.

Table 4.16 Summary of risk characterisation for workers during adhesive manufacture, assuming LEV is used

Key health effect	Human exposure	Quantitative toxicity data (body burden)	MOS	Conclusion
Acute toxicity	45 mg/kg/day (body burden)	NOAEL 235 mg/kg/day (body burden) in humans	5	ii
Irritation (skin and eye)	Low, provided good occupational hygiene practices are in operation	No quantitative data	-	ii
Repeat dose toxicity: functional CNS disturbance	45 mg/kg/day (body burden)	NOAEL 38 mg/kg/day (body burden) in humans	0.8	**iii**
Repeat dose toxicity: kidney	45 mg/kg/day (body burden)	NOAEL 65 mg/kg/day (body burden) in animals	1.5	**iii**
Mutagenicity	45 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**
Carcinogenicity	45 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**

Comparison of exposure and effects for adhesive manufacture, assuming LEV is not used

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm, equivalent to a body burden of 235 mg/kg/day. The estimated body burden from inhalation and dermal exposures combined during adhesive manufacture is 135 mg/kg/day, which is a factor of about 1.7 below the human NOAEL. An MOS of this magnitude raises concerns for human health, as it may not be sufficient to allow for variation between individuals in their sensitivity to this endpoint. Therefore **conclusion (iii)** is reached.

Irritation

The skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because exposure is low, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye, which could occur accidentally, then local damage is possible. Overall, however, **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the human NOAEL of 50 ppm equates to a body burden of 38 mg/kg/day. The body burden from combined inhalation and dermal exposure during adhesive manufacture is 135 mg/kg/day. Thus, the MOS is less than 1, which raises concerns for human health and thus **conclusion (iii)** is reached.

For kidney toxicity, the rat NOAEL of 100 ppm equates to a body burden of 65 mg/kg/day. The comparison with the body burden for adhesive manufacture of 135 mg/kg gives an MOS of less than 1, which raises concerns for human health and thus **conclusion (iii)** is reached.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. High standards of control are available in this industry sector, representing best practice for a substance with these properties. Manufacture of adhesives carried out without the use of LEV clearly does not meet the standards of control required for a substance with these properties. Therefore it is considered that risk reduction measures are required, and **conclusion (iii)** applies.

Table 4.17 Summary of risk characterisation for workers during adhesive manufacture, assuming LEV is not used

Key health effect	Human exposure	Quantitative toxicity data (body burden)	MOS	Conclusion
Acute toxicity	135 mg/kg/day (body burden)	NOAEL 235 mg/kg/day (body burden) in humans	1.7	**iii**
Irritation (skin and eye)	Low, provided good occupational hygiene practices are in operation	No quantitative data	-	ii
Repeat dose toxicity: functional CNS disturbance	135 mg/kg/day (body burden)	NOAEL 38 mg/kg/day (body burden) in humans	0.3	**iii**
Repeat dose toxicity: kidney	135 mg/kg/day (body burden)	NOAEL 65 mg/kg/day (body burden) in animals	0.5	**iii**
Mutagenicity	135 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**
Carcinogenicity	135 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**

4.1.3.2.4 Adhesive use

During use of the adhesive the workers may be exposed to the trichloroethylene vapour. Trichloroethylene is used in adhesives where a solvent of low flammability with the desired drying time is required. Applications for these adhesives are numerous, therefore it was not possible to obtain comprehensive exposure data. Exposure may be to small amounts or to high concentrations during extensive use in confined areas. It is unlikely that companies would routinely air sample for trichloroethylene during such uses, although control regimes may include local exhaust ventilation and respiratory protective equipment to reduce exposure.

Comparison of exposure and effects for adhesive use

Since there are no quantitative exposure data available it is not possible to conduct a formal comparison of exposure and health effects data. However, because high exposures are considered possible, a **conclusion (iii)** has been reached for acute toxicity and repeated dose toxicity. **Conclusion (iii)** applies for mutagenicity and carcinogenicity as it is considered that the controls currently in place in this sector of industry do not represent best practice for a substance with mutagenic and carcinogenic properties.

4.1.3.2.5 Manufacture of HCFC 133a and HFC 134a

It is estimated that about 100 workers in the EU are exposed to trichloroethylene during the manufacture of these chemicals, mainly via inhalation. Industry sampling surveys indicate 8-hour TWA exposures up to a maximum of 11.5 ppm, with a mean of about 0.2 ppm.

For use as a chemical intermediate dermal exposure was predicted using EASE to be in the range 0 to 0.1 mg/cm^2/day, although on most days no significant contacts will occur. Operators are understood to wear gloves where the potential for skin contact exists and thus in reality exposure will be towards the bottom of this range. However, taking a worst-case approach and assuming that exposure up to 0.1 mg/cm^2/day occurs, this could lead to a total estimated exposure of 80 mg/worker/day, assuming that the area of two hands (820 cm^2) is exposed. Thus, a 70 kg worker could potentially receive a body burden of about 1 mg/kg/day from dermal exposure, assuming 100% dermal absorption.

To assess the potential total body burden for workers exposed during manufacture of HCFC 133a and HFC 134a, the dermal estimates must be combined with that for inhalation exposure. For exposure to an atmospheric concentration of about 11 ppm (60 mg/m^3), assuming that 10 m^3 of air is inhaled in a working day and 100% of the inhaled dose is absorbed, a 70 kg worker could receive a body burden of up to 9 mg/kg/day. A total body burden from inhalation and dermal exposure combined could therefore be up to about 10 mg/kg/day.

For comparison with the body burden arising from workplace exposure, the quantitative rat toxicity data have been converted to an estimated body burden using the assumptions of 100% absorption by the inhalation route, 350 g bodyweight and a breathing rate of 6 l/hr. The quantitative human data have been converted to an estimated body burden using the assumptions indicated above.

See “Manufacture” above for discussion of best practice.

Comparison of exposure and effects for manufacture of HCFC 133a and HFC 134a

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm, equivalent to a body burden of 235 mg/kg/day. The highest body burden arising from combined inhalation and dermal exposure during manufacture of HCFC 133a and HFC 134a is 10 mg/kg/day, which is a factor of 23 below the human NOAEL. A MOS of this magnitude provides reassurance that adverse health effects will not occur, even allowing for variation between individuals in their sensitivity to this endpoint. Therefore **conclusion (ii)** is reached.

Irritation

The skin and eye irritation of the liquid substance is unlikely to be expressed during normal handling and use because exposure is negligible, providing good occupational hygiene practices are in operation. However, if there is contact with the skin or eye, which could occur accidentally, then local damage is possible. Overall, however, **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the NOAEL of 50 ppm equates to a body burden of 38 mg/kg. Thus, the MOS is 4, based on a body burden for combined inhalation and dermal exposure of 10 mg/kg/day. Although relatively low, this MOS is considered acceptable because the NOAEL is a precautionary estimate, based on human data, and account should be taken of the much higher animal NOAEL of 200 ppm for this endpoint, which suggests that the human NOAEL could be higher. Thus, **conclusion (ii)** is reached for this endpoint.

For kidney toxicity, the MOS is about 6, based on an animal NOAEL of 100 ppm (body burden of 65 mg/kg/day); an MOS of this magnitude raises concerns for human health as it may not be sufficient to allow for differences in toxicokinetics and toxicodynamics between and within species. Thus, **conclusion (iii)** is reached for this endpoint.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. Although high standards of control are available in this industry sector, representing best practice for a substance with these properties, there is no evidence that these standards are currently applied consistently across all EU industry. Thus, there is no evidence that the appropriate equipment is in place in all EU workplaces and that it is used and maintained in the correct manner. Therefore it is considered that risk reduction measures are required, and **conclusion (iii)** applies.

Table 4.18 Summary of risk characterisation for workers during manufacture of HCFC 133a and HFC 134a

Key health effect	Human exposure	Quantitative toxicity data	MOS	Conclusion
Acute toxicity	10 mg/kg/day (body burden)	NOAEL 235 mg/kg (body burden) in humans	23	ii
Irritation (skin and eye)	Negligible	No quantitative data	-	ii
Repeat dose toxicity: functional CNS disturbance	10 mg/kg/day (body burden)	NOAEL 38 mg/kg (body burden) in humans	4	ii
Repeat dose toxicity: kidney	10 mg/kg/day (body burden)	NOAEL 65 mg/kg in animals	6	**iii**
Mutagenicity	10 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**
Carcinogenicity	10 mg/kg/day (body burden)	Non-threshold assumed	-	**iii**

4.1.3.2.6 Summary of risk characterisation for workers

The key health effects are acute toxicity, skin and eye irritation, repeated dose toxicity, mutagenicity and carcinogenicity. For acute toxicity, a NOAEL of 300 ppm has been identified in humans for CNS depression. No quantitative data are available for skin and eye irritation. For repeated dose toxicity, a precautionary NOAEL of 50 ppm for functional CNS disturbance in humans has been identified. Additionally, a repeated dose NOAEL of 100 ppm for kidney toxicity is derived from animals. For mutagenicity and carcinogenicity it is assumed for risk characterisation purposes that there is no threshold exposure level. Workplace exposures occur during trichloroethylene manufacture and recycling, metal cleaning operations, adhesive manufacture and use, and HCFC 133a and HFC 134a manufacture.

Considering mutagenicity and carcinogenicity, it has not been possible to identify a threshold exposure level below which these effects would not be expressed and therefore there are concerns for human health at all exposures; there is no evidence that the controls currently in place across all industry sectors in the EU represent best practice for a mutagenic and carcinogenic substance, so **conclusion (iii)** applies to all scenarios. Also for all uses, the margins between exposure and the NOAEL for repeated dose kidney toxicity are of insufficient magnitude to provide reassurance that health effects will not occur, and therefore **conclusion (iii)** applies.

The margins between exposure and the NOAELs for acute CNS depression are low for metal cleaning, adhesive manufacture (without LEV) and adhesive use, and **conclusion (iii)** applies. The margins for repeat dose functional CNS disturbance also cause concern for metal cleaning, adhesive manufacture (irrespective of LEV use) and adhesive use, and **conclusion (iii)** applies. For all uses, the risk of skin and eye irritation is considered low, providing good occupational hygiene practices are in operation, and **conclusion (ii)** applies.

4.1.3.3 Consumers

The only identified consumer use for trichloroethylene in the EU is as a fabric spot cleaner. It is sold in glass bottles in many Italian supermarkets (national chains) 99.9% pure, with no instructions on use. They are placed on the same shelves with other household cleaning products. The same product has also been found in supermarkets in Belgium. It is assumed that use will be occasional and the exposure can be regarded as a one-off acute experience.

Inhalation exposure has been estimated using the US EPA *SCIES* model, with the assumption that 50 ml will be used, the cleaning lasts for 10 minutes and the person involved continues normal domestic activity in the room for the rest of the day; the model predicts exposure to a peak airborne concentration of 448 ppm (2,400 mg/m^3) and a potential body burden of 27 mg/kg for a 70 kg person. Dermal uptake for the same scenario, using the US EPA *Dermal* model, is estimated to be 35 mg/kg/day for a 70 kg person. Thus, a total body burden of 62 mg/kg for combined inhalation and dermal exposure can be estimated.

The main toxic effect of acute exposure is CNS depression. In human volunteer inhalation studies no significant effects have been seen at exposure levels of 300 ppm for between 2 and 8 hours, but impairment of performance in visual-motor tests and symptoms such as light-headedness, dizziness and lethargy have been associated with a 2-hour exposure of 1,000 ppm for 2 hours. Exposure to the human NOAEL of 300 ppm for 8 hours could result in a body burden of 235 mg/kg (assuming 10 m^3 of air is inhaled, 100% of the inhaled dose is absorbed, and the person weighs 70 kg). Thus the human NOAEL is almost 4-fold greater than the estimated human exposure.

Although this is a 4-fold MOS, and one which is reassuringly based on human hazard and dose response data, there is still considered to be cause for concern, taking into account

3. the possibility of short-term exposures above the inhalation NOAEL;
4. the steepness of the dose-response curve;
5. that the young, old or infirm persons may be exposed;
6. the potentiating effects of alcohol.

Thus there are uncertainties regarding the risks to human health at the modelled consumer exposure estimates. However, there is a suspicion that the modelled exposure estimate may be unrealistically high and consequently actual exposure data would be required to provide a risk characterisation that has adequate scientific support.

The EC's Working Group on the Classification and Labelling of Dangerous Substances has recently concluded that trichloroethylene should be classified for potential carcinogenicity (category 2) and mutagenicity (category 3). These classifications mean that the substance causes concerns for humans owing to possible mutagenic effects, and that it should be regarded as if it is carcinogenic to humans.

In view of the potential for consumer exposure in this scenario, there are concerns for human health and **conclusion (iii)** will apply for this endpoint.

It should be noted that as a result of the classification of trichloroethylene as a category 2 carcinogen, the current use of trichloroethylene in consumer products would no longer be acceptable, under existing EC legislation (Directive 76/769/EEC; Marketing and Use Directive).

4.1.3.4 Humans exposed via the environment

Environmental exposures of $1.5 \cdot 10^{-4}$ mg/kg/day for the regional model and 0.022 mg/kg/day for a local model are estimated (see Sections 3.1.6 and 4.1.1.4). The local intake is dominated by a predicted environmental concentration in air of 0.065 $mg.m^{-3}$ leading to an inhalation contribution to body burden of 0.019 mg/kg/day.

Comparison of exposure and effects

Acute toxicity

The NOAEL for CNS depression in humans for exposures of up to 8 hours is 300 ppm, equivalent to a body burden of 235 mg/kg/day. This is several orders of magnitude above the predicted body burdens arising from regional and local environmental exposures and thus does not raise concerns for human health. **Conclusion (ii)** is therefore reached for this endpoint.

Irritation

The skin and eye irritation potential relates to liquid trichloroethylene and therefore this endpoint is not relevant to environmental exposures and **conclusion (ii)** is reached.

Repeated dose toxicity

For functional CNS disturbance, the human NOAEL of 50 ppm equates to a body burden of 38 mg/kg/day. For kidney toxicity, the NOAEL of 100 ppm for the rat equates to a body burden of 65 mg/kg/day. These are at least three orders of magnitude above the predicted body burdens

arising from regional and local environmental exposures and thus do not raise concerns for human health. **Conclusion (ii)** is therefore reached for this endpoint.

Mutagenicity and carcinogenicity

These endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently **conclusion (iii)** is reached. However, the predicted regional and environmental exposures are very low. Therefore, although there may be some residual risk of mutagenicity and/or carcinogenicity this is likely to be very low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

4.1.3.5 Combined exposure

The potential combined exposure will be dominated by the occupational exposure, particularly if marketing and use restrictions are applied to the use of trichloroethylene in consumer applications. Thus, the conclusions of the risk characterisation for combined exposure will reflect those reached for workers.

4.2 HUMAN HEALTH (PHYSICOCHEMICAL PROPERTIES)

Trichloroethylene would not be classified as flammable and it is unlikely to be flammable except in exceptional circumstances, perhaps where vapour is contained in a sealed vessel and exposed to high-energy ignition sources.

Trichloroethylene is not considered to be explosive. However, violent decomposition is possible under certain conditions in the presence of aluminium. Commercial grades have stabilisers added to prevent such reactions in normal use and storage.

Further assessment of the risk arising from physicochemical properties relates to local controls for storage and use and is best addressed at that level.

Overall, **conclusion (ii)** is reached.

5 RESULTS

5.1 INTRODUCTION

Trichloroethylene is made by four companies within the EU, with an estimated production tonnage of 138,000 tonnes in 1996. Of this, 77,000 tonnes were sold within the EU, for use mainly in metal cleaning with a range of other minor uses (including adhesives and consumer cleaning products). Some of the remaining 61,000 tonnes was exported, and it is estimated that ~41,000 tonnes are used as an intermediate. The quantities produced and used and the distribution between them may have changed in recent years.

5.2 ENVIRONMENT

The environmental risk characterisation considers the production of trichloroethylene and its use as an intermediate, formulation as a solvent, in metal cleaning, formulation and use in adhesives and consumer products, and 'other' uses. It also considers the potential formation and effects of a breakdown product, dichloroacetic acid.

For the aquatic compartment the PEC/PNEC ratios are less than one for all of the life cycle steps considered for water and sediment. The ratios for wastewater treatment plants are also less than one. For dichloroacetic acid, the concentrations in surface waters are expected to be less than the PNEC.

For the terrestrial compartment, the PEC/PNEC ratios are less than one for trichloroethylene exposure in soil (through sewage sludge application and aerial deposition). The concentrations of dichloroacetic acid in soil measured at a range of sites are lower than the PNEC estimated for dichloroacetic acid, which indicates no concern.

This assessment does not address risks arising from groundwater contamination.

For the atmospheric compartment, some measured or calculated concentrations in air are higher than the PNEC for plants exposed through the air (the PNEC value was extrapolated from that for tetrachloroethylene). The areas concerned are production, use as an intermediate, formulation as a solvent and metal degreasing.

The assessment of secondary poisoning gives PEC/PNEC ratios less than one for all life cycle steps considered.

Results

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

This conclusion applies to the risk of harm to plants from air emissions of trichloroethylene from sites producing trichloroethylene, from sites using trichloroethylene as an intermediate, from sites formulating trichloroethylene as a solvent ("handling"), and from use in metal degreasing. The conclusion for production applies to two sites, and the conclusion for intermediate use applies to sites which have not provided emission information.

The PNEC on which this conclusion is based is derived from that for tetrachloroethylene on the basis that both substances would be expected to have similar effects. The conclusions of the

human health risk assessment will require risk reduction action to be taken for this substance. The Solvent Emissions Directive (1999/13/EC) will also have an impact on the emissions of this substance, in particular on use in metal degreasing.

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

This conclusion applies to the aquatic compartment (including sediment), to wastewater treatment plants, to the terrestrial environment and to secondary poisoning for all stages in the production and use of trichloroethylene; to the atmospheric compartment for adhesive formulation and use, consumer product formulation and use, and 'other' uses; and to the aquatic and terrestrial compartments for dichloroacetic acid produced by the photodegradation of trichloroethylene.

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

Studies in experimental animals and humans have shown that trichloroethylene is rapidly and extensively absorbed by all routes of exposure. Human experience and limited animal experimental data indicate that liquid trichloroethylene is a skin and eye irritant. There are no concerns for sensitisation.

The main toxic effect associated with acute inhalation exposure is CNS depression. Exposure to very high concentrations causes narcosis. However extensive experience in the use of trichloroethylene as an anaesthetic has demonstrated that recovery from narcosis is usually complete.

The repeated dose toxicity of trichloroethylene in humans has not been well characterised, although there is an extensive database. Overall, based on the available information, functional CNS disturbance is regarded as a key endpoint in humans, although the available data do not allow conclusive judgements to be made regarding causal or dose-response relationships. Notwithstanding the dose-response uncertainties, the overall view reached by the majority of Member States in discussing this endpoint is that there appears to be an absence of CNS effects associated with exposure levels of around 50 ppm and taking a cautious approach, this value was identified as a NOAEL for functional CNS disturbance. However, it is noted that in animal studies, a clear NOAEL for neurotoxicity of 200 ppm has been identified for long-term inhalation exposure to trichloroethylene. In view of this, and given the uncertainties surrounding the identification of a reliable NOAEL for humans from the available data, it is possible that NOAEL for neurotoxicity in humans may be higher than 50 ppm.

Kidney toxicity appears to be the most sensitive endpoint for both long-term repeated inhalation and oral exposure in animals. While there are no reports of trichloroethylene-related kidney toxicity in the human studies, this endpoint has not been properly investigated in humans. Therefore, because of the paucity of human data for this endpoint, the animal data have been considered in the risk characterisation, and a NOAEL of 100 ppm is identified for kidney toxicity.

The genotoxicity of trichloroethylene has been extensively investigated in experimental test systems. Trichloroethylene tested positive in a bacterial (Ames) test and a mouse lymphoma gene mutation assay, demonstrating that trichloroethylene is an *in vitro* mutagen. There is

contradictory evidence of *in vivo* genotoxic activity and consequently there have been uncertainties about whether trichloroethylene should be classified for potential mutagenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity. The majority of the members of this group concluded, based on a weight of evidence assessment approach, that the positive evidence for DNA interaction justified classification as a category 3 mutagen. This indicates that, in the opinion of the majority of Specialised Experts, the substance causes concerns for humans owing to possible mutagenic effects. In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly.

The carcinogenicity of trichloroethylene has been investigated in occupationally exposed populations in a number of studies. The majority of these did not show any evidence that trichloroethylene exposure is associated with an increased incidence of cancer. Individually, each of the negative studies had certain limitations although several, by nature of their design, had substantial power to detect an effect, and when taken together provide significant evidence that trichloroethylene is not carcinogenic in humans under the exposure conditions experienced by the groups studied. However, the occupational studies did not provide complete reassurance of the absence of trichloroethylene-related carcinogenicity in humans. This was because limited evidence of an increased risk of cancer among trichloroethylene exposed workers was reported in one well-conducted cohort study, although definitive conclusions about causality could not be made in view of the limited corroboration with the results of the other studies. Furthermore, in two other studies an increased risk of kidney cancer was reported in a group of trichloroethylene workers; although it was not possible to draw any conclusion from these studies because of methodological weaknesses, this association adds to the concerns about the carcinogenic potential of trichloroethylene. Overall, given the observation of kidney tumours in animal studies and absence of convincing evidence that these arose by mechanisms not applicable to humans, together with the uncertainties regarding the human data, it is considered that there remains some concern for potential carcinogenicity in humans.

There have been uncertainties about whether trichloroethylene should be classified for potential carcinogenicity. In March 2000, this issue was referred to an EC Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity. The majority of the members of this group concluded that classification as a category 2 carcinogen is warranted, based on evidence of carcinogenicity in one animal species, namely tumours in the rat kidney, supported by epidemiological data showing an association between exposure and kidney tumours and non-Hodgkin's lymphoma in humans. This indicates that, in the opinion of the majority of Specialised Experts, trichloroethylene should be regarded as if the substance is carcinogenic to humans. In April 2000, the EC's Working Group on the Classification and Labelling of Dangerous Substances decided to accept the recommendation made by the Specialised Experts and it was agreed that the existing classification and labelling of trichloroethylene would need to be revised accordingly.

It is not possible to draw any firm conclusions about the reproductive toxicity of trichloroethylene on the basis of the available human data; accordingly, the risk characterisation for reproductive effects must be based on animal data. Effects of long-term oral administration on fertility and reproductive performance have been extensively investigated in rats and mice. Trichloroethylene was shown to have some influences on reproduction but only at exposure levels that produce general toxicity in adult animals. The observed effects included reduced sperm motility and reductions in neonatal bodyweight and survival in mice at high-dose levels

and, in rats, disrupted copulatory behaviour and reduced pup survival at high-dose levels, and reductions in the litter size and number of litters born to continuously bred animals. NOAELs for reproductive effects with oral dosing were 350 mg/kg/day in mice and 75 mg/kg/day in rats. Two studies in mice have suggested that short-term repeated trichloroethylene exposure can influence sperm morphology; a NOAEL for this effect was 200 ppm. However, effects on sperm morphology were not seen in rats, nor in large-scale studies in which mice received long-term exposure to high levels of trichloroethylene by the oral route. The reproductive NOAELs have not been considered in the risk characterisation because these are greater than the NOAELs for repeated dose toxicity. The developmental toxicity of inhaled trichloroethylene at non-maternally toxic levels (up to 1,800 ppm) has been investigated in rats, mice and rabbits in conventional studies. No evidence of developmental toxicity was reported. In contrast, the results of a series of non-standard oral studies in rats raise concerns about the potential for trichloroethylene to induce developmental neurotoxicity at dose levels in the range of 30-110 mg/kg/day. However, these studies were of limited scope and were considered not to provide sufficient basis on which to draw clear conclusions about the hazardous properties of trichloroethylene. To be able to draw clear conclusions regarding developmental neurotoxicity, further testing according to the draft OECD TG 426 would be required. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen the results of such testing are unlikely to influence the outcome of the risk assessment. This is because the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

5.3.1.1 Workers

The highest human exposures are in the workplace. The majority of measurements of personal exposures to airborne trichloroethylene in manufacturing were low, with significant excursions which would be controlled by the use of respiratory protective equipment. Inhalation exposures for recycling and manufacture of HFCs and HCFCs were similarly low, although based on very little information. Inhalation exposures in dry cleaning were somewhat higher. Under certain circumstances, there may be additional exposure via the dermal route, although this may be readily controlled with appropriate protective clothing.

More people are potentially exposed via metal degreasing than any other industry. While exposures can be controlled to a low level, the results of short-term measurements indicate that exposures during loading and unloading of degreasing baths may be considerably higher; these raised short-term exposures may result in high shift exposures. Cleaning of the degreasing bath may lead to yet higher exposures, although personal exposures to these levels may be controlled (and reduced) by the use of breathing apparatus. No information on dermal exposure is available but trichloroethylene is rapidly and extensively absorbed adding significantly to the total body burden.

Results

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

In relation to concerns for acute CNS depression, exposures occurring during metal cleaning, adhesive manufacture (without LEV) and adhesive use are considered to give rise to concern and conclusion (iii) is reached. The margins for repeat dose functional CNS disturbance also cause concern for metal cleaning, adhesive manufacture (irrespective of LEV use) and adhesive use, and conclusion (iii) applies. For all uses, the margins between exposure and the NOAEL for

repeated dose kidney toxicity are of insufficient magnitude to provide reassurance that health effects will not occur, and therefore conclusion (iii) applies.

In relation to mutagenicity and carcinogenicity, these endpoints have no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels and consequently conclusion (iii) is reached. Although high standards of control are available in all industry sectors, representing best practice for a substance with these properties, there is no evidence that these standards are currently applied consistently across all EU industry. Thus, there is no evidence that the appropriate equipment is in place in all EU workplaces and that it is used and maintained in the correct manner. Therefore it is considered that risk reduction measures are required, and conclusion (iii) applies.

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

For all uses, the risk of skin and eye irritation is considered low, providing good occupational hygiene practices are in operation, and conclusion (ii) applies.

5.3.1.2 Consumers

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

Consumer exposure is limited to intermittent use of fabric "spot" cleaners. The EC's Working Group on the Classification and Labelling of Dangerous Substances has recently concluded that trichloroethylene should be classified for potential carcinogenicity (category 2) and mutagenicity (category 3). These classifications mean that the substance causes concerns for humans owing to possible mutagenic effects, and that it should be regarded as if it is carcinogenic to humans. In view of the potential for consumer exposure, there are concerns for human health as a result of this consumer use of trichloroethylene and conclusion (iii) will apply for this endpoint.

It should be noted that as a result of the classification as a category 2 carcinogen, the current use of trichloroethylene in consumer products would no longer be acceptable, under existing EC legislation (Directive 76/769/EEC; Marketing and Use Directive).

5.3.1.3 Humans exposed via the environment

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

For carcinogenicity and mutagenicity endpoints, there is no identifiable threshold exposure level below which the effects would not be expressed, so there are health concerns at all exposure levels. However, the predicted regional and environmental exposures are very low. Therefore, although there may be some residual risk of mutagenicity and/or carcinogenicity this is likely to be very low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

It is not possible to draw clear conclusions regarding developmental neurotoxicity. Further testing according to OECD TG 426 is needed. However, as the substance is classified as a category 3 mutagen and a category 2 carcinogen, the results of such testing are unlikely to

influence the outcome of the risk assessment, as the risk characterisation is based on the assumption that a threshold exposure level for adverse health effects cannot be identified.

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

In relation to acute CNS effects, skin and eye irritation, repeated dose functional CNS disturbance and repeated dose kidney toxicity, there is no significant risk for humans exposed via environmental routes.

5.3.1.4 Combined exposure

The potential combined exposure will be dominated by the occupational exposure, particularly if marketing and use restrictions are applied to the use of trichloroethylene in consumer applications. Thus, the conclusions of the risk characterisation for combined exposure will reflect those reached for workers.

5.3.2 Human health (risks from physico-chemical properties)

Trichloroethylene would not be classified as flammable and it is unlikely to be flammable except in exceptional circumstances, perhaps where vapour is contained in a sealed vessel and exposed to high-energy ignition sources.

Trichloroethylene is not considered to be explosive. However, violent decomposition is possible under certain conditions in the presence of aluminium. Commercial grades have stabilisers added to prevent such reactions in normal use and storage.

Results

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

If the appropriate conditions of handling and storage are adhered to, there are no concerns for risks to human health arising from the physicochemical properties of trichloroethylene.

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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
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
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
CEPE	European Committee for Paints and Inks
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
foc	Organic carbon factor (compartment depending)
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)
OC	Organic Carbon content
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
PBT	Persistent, Bioaccumulative and Toxic

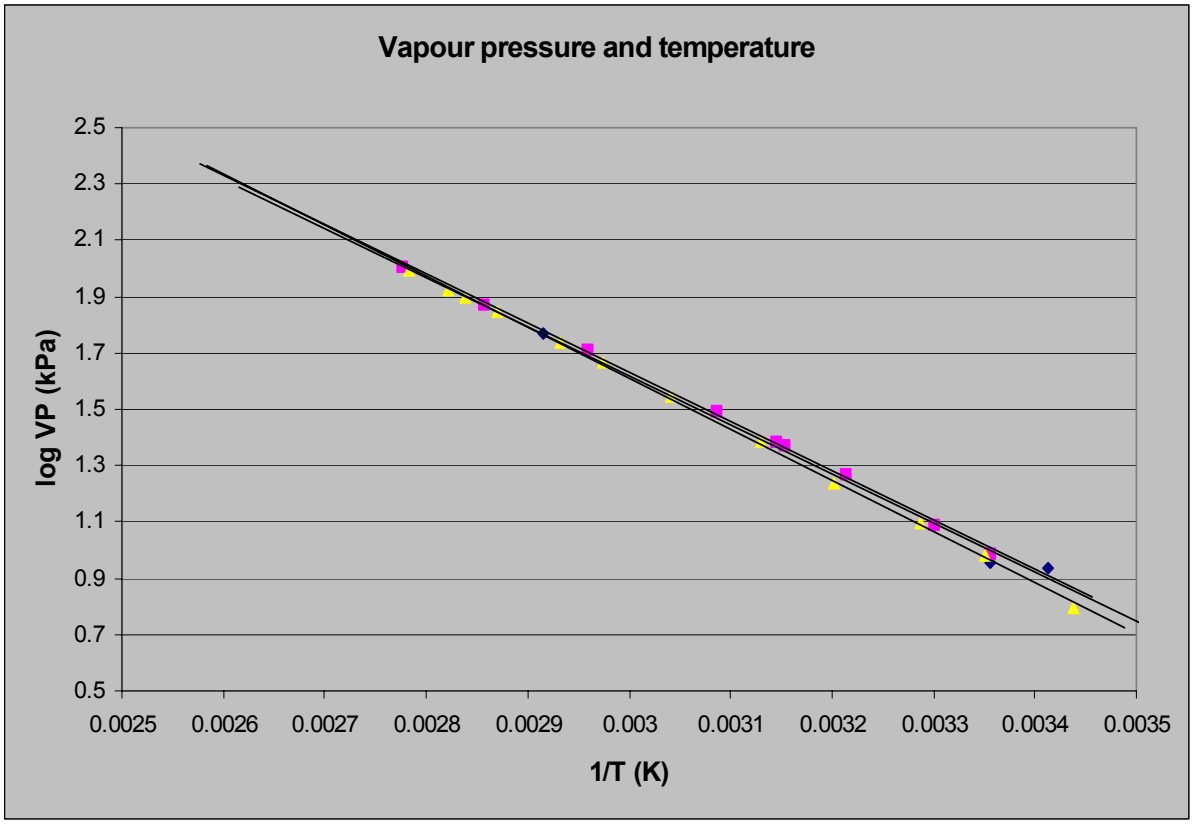
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to 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
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
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
VOC	Volatile Organic Compound
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	Waste Water 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)

Appendix A Vapour pressure for trichloroethylene

McDonald (1944) (▲)		IUCLID data (◆)		Hertz et al (1912) (■)	
Temperature (°C)	Vapour pressure (kPa)	Temperature (°C)	Vapour pressure (kPa)	Temperature (°C)	Vapour pressure (kPa)
17.8	6.24	20	8.6	25	9.73
25.5	9.55	25	9.08	30	12.27
31.2	12.43	70	59	38.2	18.53
39.29	17.31			44.15	23.6
46.44	24.32			45	24.4
55.86	35.21			51	31.2
63.27	46.2			65	51.33
68.05	54.41			77	74.93
75.49	69.95			87.15	101.33
79.22	79.07				
81.31	84.23				
86.32	98.93				

McDonald (1944) (▲); Hertz et al. (1912) (■); IUCLID (◆)



Appendix B Risk assessment for the breakdown products from degradation of trichloroethylene in air (dichloroacetic acid)

Section 3.1.2.1.1 of the main risk assessment report discusses the possible products from the photodegradation of trichloroethylene in the atmosphere. One of the potential products is dichloroacetic acid (DCA), which may be of concern due to its anticipated herbicidal properties. This Appendix contains estimates of the amount of DCA which could be formed from the breakdown of trichloroethylene, and the levels of DCA in the environment which could result from this. An assessment of the potential toxicity of DCA is also presented. A risk characterisation is then carried out based on the DCA formed from trichloroethylene.

Dichloroacetic acid is not a major industrial chemical and there are not expected to be significant releases from this area. However a further possible source is formation during the chlorination of water, and this is discussed later in this Appendix.

Exposure assessment

Estimation of the production of dichloroacetic acid from trichloroethylene

(In these notes, radical species are not marked as such)

Rate constants for initial reactions

The two initial reactions of trichloroethylene are with hydroxyl radicals or chlorine atoms.

Rate constant for reaction with OH radicals: $2.36 \cdot 10^{-12}$ $cm^3 \cdot molec^{-1} \cdot s^{-1}$ (Atkinson, 1985)
Rate constant for reaction with chlorine atoms: $8.1 \cdot 10^{-11}$ $cm^3 \cdot molec^{-1} \cdot s^{-1}$ (Atkinson and Aschmann, 1987).

Taking the TGD hydroxyl radical concentration of $5 \cdot 10^5$ $molec \cdot cm^{-3}$, and the chlorine atom concentration as 500 $molec \cdot cm^{-3}$ (Aucott, 1997) then the rate constants are:

k[OH]	$1.18 \cdot 10^{-6}$ s^{-1}	96.7%
k[Cl]	$4.05 \cdot 10^{-8}$ s^{-1}	3.3%

These values together correspond to a lifetime of 9.5 days, 0.026 years.
[The accepted lifetime of trichloroethylene appears to be 0.018 years.]

Reaction of trichloroethylene with chlorine atoms involves addition of the Cl atom. There are two sites for this addition as follows:

$$Cl + CHCl{=}CCl_2 \rightarrow CHCl_2CCl_2 \qquad 1a$$

$$Cl + CHCl{=}CCl_2 \rightarrow CCl_3CHCl \qquad 1b$$

Two studies on these reactions show that reaction 1a predominates; Bertrand et al. (1968) found the ratio 1a:1b to be >8, while Catoire et al. (1997) concluded that reaction 1a represented (100±12)% of the overall addition, i.e. 1a:1b >7.3. As a worst case it will be assumed that all the reaction proceeds via 1a.

Subsequent reaction steps

Subsequent reactions of the radical produced in 1a are:

$$CHCl_2CCl_2 + O_2 \rightarrow CHCl_2CCl_2O_2$$
$$CHCl_2CCl_2O_2 + NO \rightarrow CHCl_2CCl_2O$$

There are two routes by which this last radical can react:

$$CHCl_2CCl_2O \rightarrow CHCl_2COCl + Cl \quad 2a$$

$$CHCl_2CCl_2O \rightarrow CHCl_2 + CCl_2O \quad 2b$$

The product of reaction 2a is dichloroacetyl chloride, DCAC. Reaction 2b leads to one-carbon products (formyl chloride and phosgene). Three estimates of the relative rates of these two reactions are available. Bertrand et al. (1968) measured the ratio 2a:2b as (85±3):(15±3); Catoire et al. (1997) came to a similar conclusion, (91±11):(9±2). The most recent determination (Hasson and Smith, 1999) gives the product ratio as (91±3):(9±1). Taking the mean value gives 2a:2b as 89:11.

The DCAC from reaction 2a can hydrolyse in clouds and rainwater, or can undergo photolysis to one-carbon products:

$$DCAC + H_2O \rightarrow DCA + HCl \quad 3a$$

$$DCAC + h\nu \rightarrow C_1 \text{ products} \quad 3b$$

The lifetime of DCAC for reaction with water is likely to be similar to that for TCAC, namely 20 days (Kindler et al., 1995). The lifetime with respect to photolysis is given as 9 days by WMO (1994). Therefore the ratio of the two processes hydrolysis:photolysis is 31:69.

The overall yield of dichloroacetic acid as a percentage of the chlorine atom reaction pathway is the product of the yields from the reaction steps above, ie $1 \cdot 0.88 \cdot 0.31 = 0.28$ (28%).

From the relative reaction rates for OH and Cl addition, the Cl pathway accounts for 3.3% of trichloroethylene. Hence the overall yield of dichloroacetic acid based on trichloroethylene is 0.9%.

This calculation is intended as a rough estimate of the possible formation of dichloroacetic acid. Not all of the rates above have been measured with a high degree of certainty; this is particularly true for the hydrolysis and photolysis rates of DCAC.

Concentrations estimated from anthropogenic emissions

Anthropogenic emissions of trichloroethylene have been estimated for the Northern and Southern hemispheres (McCulloch and Midgley, 1996). As the lifetime of trichloroethylene in the atmosphere is considerably shorter than the interhemispheric mixing time (around 1 year) then there should be no movement of these emissions between the hemispheres. The most recent estimates for trichloroethylene releases are for 1992: 197,000 tonnes in the Northern hemisphere, and 2,000 tonnes in the Southern.

From the estimated yield of dichloroacetic acid of 0.9%, these releases would give 1,740 t (N) and 18 t (S). Taking the total rainfall in each hemisphere as $2.5 \cdot 10^{14}$ m^3 each year would give concentrations of 7 and 0.07 ng/l respectively. These values are below those observed at remote locations in precipitation by von Sydow (1998) (see below), over 2 orders of magnitude lower in the case of the Southern hemisphere values.

Concentrations based on calculated total flux

Section 3.1.1.3 in the main assessment describes studies by McCulloch and Midgley (1996) and Aucott (1997) which conclude that a large natural source of trichloroethylene is required to reconcile the measured concentrations in the atmosphere at remote locations and the known rate of reaction with hydroxyl radicals.

Taking the average concentrations in each hemisphere as 3 pptv (N) and 0.6 pptv (S) (Koppmann et al., 1993) and taking the lifetime derived from the OH and Cl reaction rates described above (0.026 years) gives the fluxes of trichloroethylene in each hemisphere as $1.26 \cdot 10^6$ tonnes/year (N) and $2.52 \cdot 10^5$ tonnes/year (S). These are clearly larger than the anthropogenic releases estimated above.

Using the above fluxes and assuming a yield of 0.9% for dichloroacetic acid gives annual production of dichloroacetic acid as $1.1 \cdot 10^4$ tonnes (N) and $2.2 \cdot 10^3$ tonnes (S). Taking the annual rainfall in each hemisphere as $2.5 \cdot 10^{14}$ m^3 gives concentrations of dichloroacetic acid in rain of 44 ng/l (N) and 9 ng/l (S). These values agree much more closely with those measured by von Sydow (1998) in remote locations (10-70 ng/l for the Northern hemisphere, 10-20 ng/l for the Southern).

Measured levels of dichloroacetic acid

There are only a few measured levels of dichloroacetic acid available.

Levels in precipitation

Levels of dichloroacetic acid have been measured in remote regions in snow, ice and rain (von Sydow, 1998). These measurements indicate current and historical levels. A summary of their findings is given in **Table B.1**.

Table B.1 Levels of dichloroacetic acid in remote samples

Location	Level (ng/l)
Pre-industrial Swedish ice	12
Current snow in Norway and Sweden	10 - 30
Snow on Russian tundra	20 - 70
Snow from British Columbia	10 - 30
Contemporary snow from Dronning Maud Land, Antarctica	10 - 20
Older Antarctic firn	9 - 26

These show that dichloroacetic acid is ubiquitous in precipitation around the world. The older samples from Sweden and those in Antarctic firn indicate that dichloroacetic acid was present before the large-scale anthropogenic release of trichloroethylene.

Schleyer et al. (1996) measured dichloroacetic acid in rainwater in south-western Germany, at sites in spruce forests, beech woods, mixed woods and on open land. Their results are in **Table B.2**.

Table B.2 Levels of dichloroacetic acid in rainwater in south-western Germany

	No of samples	min value	median	max value
open land	115	<0.035	<0.035	0.254
spruce forest	46	<0.035	0.045	0.20
beech wood	34	<0.035	0.052	0.33
mixed wood	21	<0.035	<0.035	0.12

Units are μg/l, converted from nmol/l in the original paper.

Reimann et al. (1996) measured dichloroacetic acid in rainfall in Zurich, summer 1993, up to 3 μg/l, and in 1994 at a mean monthly average of 1.2 μg/l.

Haiber et al. (1996) reported levels of dichloroacetic acid in rainwater collected near the source of the river Sieg as <100 - 900 ng/l, and at a second site at Hau as 50-150 ng/l.

Juuti (1997) reported levels of dichloroacetic acid in precipitation in Finland; the mean level was 240 ng/l from monthly samples over a 20-month period. Levels in snow tended to be lower than those in rain but the difference was not significant statistically.

Peters (2000) measured levels of DCA in wet deposition at two locations in The Netherlands; the concentrations found were 0.17 and 0.11 μg/l.

Levels in surface and groundwater

There are no background measurements of dichloroacetic acid in surface waters. A possible source of dichloroacetic acid in water is through chlorination. LeBel et al. (1997) showed the formation of DCA during three types of chlorination treatment and the presence of DCA in the subsequent water distribution system. The levels found were 15.7-18.3 μg/l (mean of 12 monthly samples) at the treatment sites. The concentration tended to decrease with increasing distance from the chlorination site, showing degradation of DCA. The authors commented that it was not clear if this was biotic or abiotic degradation (when DCA was discussed for classification it was agreed to be readily biodegradable but the data source for this has not been seen). Kakatuni et al. (1995) measured DCA in rivers and estuaries in Japan. Levels were highest in rivers flowing through populated and industrialised areas; and were attributed to direct discharges of domestic and industrial wastewaters. In rivers flowing through Osaka City the levels were 5-10 μg/l. It is not possible to quantify the production of DCA by this route. As DCA is ionised under normal environmental conditions (pKa = 1.3) then it is not expected to volatilise to any great extent. Therefore this route of production seems more likely to contribute to surface water levels than to those in rainwater.

Schleyer et al. (1996) also measured levels of dichloroacetic acid in percolating soil water and in ground and spring water. In percolating water, the majority of results lay below 130 ng/l (1 nmol/l in original), but four of the sites had higher levels up to 516 ng/l (4 nmol/l). These values were higher than the corresponding rainwater levels, and were attributed to breakdown of trichloroacetic acid. In groundwater the majority of levels were again below 130 ng/l, with the highest measurement being 190 ng/l.

Juuti (1997) also measured the concentration of dichloroacetic acid in one groundwater sample, at ~10 ng/l. In contrast, one sample of chlorinated tap water had a concentration of 2,660 ng/l.

In summary, remote concentrations of dichloroacetic acid are of the order of 10-20 ng/l. Mean values in rural areas available range from 50-240 ng/l. One set of measurements in Switzerland had mean levels of 1.2 μg/l. All these measurements refer to rainwater. The few measured levels in surface and drinking water have been attributed to formation through chlorination.

Levels in soil

In a recent study Peters analysed soil samples from 10 locations across Europe. Most of the sites were selected randomly, but locations in the Black Forest (Freudenstadt) and in The Netherlands (Apeldoorn) were included to compare results with earlier measurements of trichloroacetic acid. At each location, samples were taken from forest sites (i.e. beneath the tree canopy) and from nearby open sites. Samples were taken at three depths at each location. The individual results are presented in **Table B.3**; average concentrations over the depth profile and a comparison between the results from forest sites and open sites are in **Table B.4**.

Table B.3 Levels of DCA in EU soils (Peters, 2000)

Country	Area	Forest		Open	
		Depth (m)	Concentration (μg/kg dw)	Depth (m)	Concentration (μg/kg dw)
Germany	Freudenstadt	0.1 0.3 0.6	0.85 0.44 0.32	0.1 0.3 0.9	0.31 0.12 0.13
	Kiel	0.1 0.3 0.8	0.71 0.36 0.17	0.1 0.3 1.0	0.19 0.46 0.18
The Netherlands	Apeldoorn	0.1 0.3 1.0	0.45 0.13 0.15	0.1 0.3 1.0	0.18 0.20 0.15
	Rotterdam	0.1 0.3 1.0	0.16 <0.05 <0.05	0.1 0.3 0.8	0.11 0.25 0.11
Italy	Venice	0.1 0.3 0.8	0.27 0.28 0.17	0.1 0.3 0.8	0.19 0.13 0.13
	Rome	0.1 0.3 1.0	0.60 0.26 0.25	0.1 0.3 0.8	<0.05 0.10 0.13
Scandinavia	Göteborg	0.1 0.3 0.7	0.47 0.13 0.18	0.1 0.3 0.8	0.21 0.23 0.22
	Oslo	0.1 0.3 0.6	0.54 0.71 0.16	0.1 0.3 0.8	<0.05 0.11 0.06
UK	Nottingham	0.1 0.3 0.7	0.76 0.15 0.19	0.1 0.3 0.8	0.11 0.19 0.17
	Glasgow	0.1 0.3 0.6	0.30 0.31 0.13	0.1 0.3 0.8	0.12 0.13 0.17

There is a general tendency for the concentrations to decrease with depth at many of the sites, although there are also examples of increased concentrations in the second depth samples. These tendencies are much less pronounced in the open site samples, where levels are in general lower than those from the forest sites and tend to be similar down through the profile.

Table B.4 Average levels of DCA in soil (Peters, 2000)

Country	Area	Average DCA conc (µg/kg dw)		Ratio
		Forest	Open	(forest/open)
Germany	Freudenstadt	0.54	0.18	2.9
	Kiel	0.41	0.27	1.5
The Netherlands	Apeldoorn	0.24	0.18	1.4
	Rotterdam	0.16	0.16	1.0
Italy	Venice	0.24	0.15	1.5
	Rome	0.37	0.11	3.2
Scandinavia	Göteborg	0.26	0.22	1.2
	Oslo	0.47	0.08	5.6
UK	Nottingham	0.37	0.16	2.4
	Glasgow	0.25	0.14	1.7

The overall average concentration in European soil was 0.25 µg/kg.

Levels in biota

Peters (2000) sampled needles or leaves from the forest areas where soil samples were taken as described in the section "Levels in soil". In the majority of cases the levels in the biota were 10-20 times higher than the corresponding soil levels. One very high value of 73 µg/kg was found for the Freudenstadt site, which did not appear to relate to the soil levels. Other values were ≤15 µg/kg, and at two sites DCA could not be quantified due to interferences. The overall average value was 14 µg/kg dry weight.

EUSES modelling

The EUSES model was run for dichloroacetic acid with an input to the regional compartment of 50 tonnes/year to air, and 435 tonnes/year to air to the continental compartment. These were derived from the releases of trichloroethylene estimated in the main risk assessment report (Section 3.1.1.4) with a yield of DCA of 0.9%. The properties of dichloroacetic acid used were as follows:

log Kow	-0.14 (Verschueren, 1983)
solubility	86,300 mg/l (Verschueren, 1983)
vapour pressure	7.9 Pa (estimated with ChemEst)
biodegradability	readily biodegradable (based on classification, but data not seen)

The resulting concentrations on the regional scale were:

air	2.4 ng/m^3
surface water	59 ng/l
natural soil	0.1 μg/kg

There are problems with using the model for this substance, because the property values available are for the neutral form of the acid. Under environmental conditions dichloroacetic acid will be almost completely ionised, and its behaviour may differ significantly. Therefore the modelling results should only be treated as indicative. The concentration in air (which is probably the least affected by these problems) agrees well with the limited measured values available (0.6-11.5 ng/m^3 in the Black Forest in the early 1990s).

Calculation of soil levels which could result from aerial deposition of dichloroacetic acid

The following calculation estimates the concentrations in soil which could come from dichloroacetic acid deposited from the atmosphere. It uses the measured levels of DCA in rainwater, and assumes a steady state between input and removal.

From the TGD, standard rainfall is 700 mm/year, or 1.92 mm/day. On an area of 1 m^2 this is a volume of $1.92 \cdot 10^{-3}$ m^3.

For the concentration in rain, the level recently measured in The Netherlands is used, as there are contemporary measurements of the levels in soil at locations nearby. The higher measurement was 0.17 μg/l at Apeldoorn. For a concentration of 170 ng/l in rain, the amount deposited per day = 0.33 μg/m^2.

The removal half-life for biodegradation in soil is estimated using the TGD methodology. Assuming that DCA is readily biodegradable the half-life is 30 days (k = 0.023 d^{-1}).

Assuming steady state, removal rate = deposition rate
or $0.023 \cdot \text{amount} = 0.33$ μg
amount of dichloroacetic acid at steady state = 14 μg.

It is assumed that this amount is mixed into the top 10 cm of soil, in order to compare with the measured levels. The volume of soil involved is 0.1 m^3.

Assuming the standard soil properties as in the TGD, soil density = 1,700 kg/m^3
so soil mass = 170 kg.

Hence the concentration of dichloroacetic acid in soil = 14/170 ~ 0.08 μg/kg.

This calculation does not include leaching. From EUSES, the leaching rate is 0.033 d^{-1} for agricultural soil. Including this removal rate gives a soil concentration of 0.035 μg/kg.

The measured concentration at the Apeldoorn site in The Netherlands (top 10 cm) is 0.18 μg/kg dw, or 0.15 μg/kg wet weight using the measured dry weight of the soil sample for correction. The level measured at the open site is used as the measurements on DCA concentrations in rain were from open sites. The calculated levels are two or five times lower than the measured value, which could indicate that removal is not as rapid as has been estimated here.

A similar calculation for the other site in The Netherlands where the level in rainfall was measured gives a calculated level in soil of 0.05 μg/kg (biodegradation only) or 0.02 μg/kg (biodegradation and leaching), compared to 0.09 μg/kg as measured in the top 10 cm.

Other older measurements on DCA in rainwater appear to be higher in general - 240 ng/l for Finland, 1.2 μg/l for Switzerland in 1994. Using the value from Switzerland and assuming the standard EU soil as in the TGD gives a concentration in soil from rainwater deposition of 0.59 μg/kg dw, or 0.67 μg/kg wet weight, which is similar to the highest levels found in the top 10 cm in the Peters (2000) study.

Effects of dichloroacetic acid

Little information on the effects of dichloroacetic acid on the environment has been found. Frank et al. (1994) tested the effects of substances on bean cell suspension cultures to assess phytotoxicity. In a 15-day study the EC_{50} for dichloroacetic acid was 1,200 μmol/l (155 mg/l) and the EC_{10} was 400 μmol/l (52 mg/l). These results would indicate that dichloroacetic acid was not very toxic to plant cells. However trichloroacetic acid was also tested in the same assay and showed a slightly higher toxicity (values around half those for dichloroacetic acid). Trichloroacetic acid has been shown to have effects on algae at much lower levels; the OECD SIDS assessment identifies a NOEC of 8.7 μg/l and derives a PNEC of 0.17 μg/l. Dichloroacetic acid has been classified as R50 which means acute toxicity of less than 1 mg/l. Monochloroacetic acid also has a high toxicity to algae.

In order to make an estimate of the toxicity of dichloroacetic acid the available data on trichloroacetic acid and monochloroacetic acid have been compared (**Table B.5**). The data on trichloroacetic acid come from the draft SIAR; the data for monochloroacetic acid come from a UBA report "Berwertung der Umweltgefährlichkeit ausgewählter Altstoffe durch das Umweltbundesamt", Teil II, Texte 38/96 and the draft ESR Risk Assessment Report prepared by The Netherlands, February 2002.

Table B.5 Toxicity data for mono- and tri-chloroacetic acids

Species	Effect	TCA	MCA
Fish	short term (LC_{50})	2,000 mg/l	145 mg/l
	long term (NOEC)	7 mg/l	12.5 mg/l
Invertebrates	short term (LC_{50})	2,000 mg/l	77 mg/l
	long term (NOEC)		32 mg/l
Algae	EC_{50}	264 μg/l	25 μg/l
	NOEC	8.7 μg/l	5.8 μg/l

Some of the values for trichloroacetic acid are adjusted to convert from the sodium salt to the acid.

From the data monochloroacetic acid is as toxic as or more toxic than trichloroacetic acid for all endpoints where values are available for both substances. Algae are clearly the most sensitive organisms by at least three orders of magnitude. The effect responsible for the much lower long-term value in fish with trichloroacetic acid is probably irritation of the gills.

Assuming that the toxicity of dichloroacetic acid will be between that of the other two substances gives a NOEC for algae of 7.2 μg/l (mean of the two values). [The no-effect concentrations on a molar basis are 53 nmoles/l for trichloroacetic acid and 61 nmoles/l for monochloroacetic acid; the average of these is 57 nmoles/l, which corresponds to 7.4 μg/l.]

As the toxicity to algae is so much higher than to other species, a factor of 10 is applied to the NOEC so that the PNEC for dichloroacetic acid is 0.72 μg/l.

There are no data on effects on soil organisms. Using the equilibrium partitioning method would give a $PNEC_{soil}$ of 0.11 μg/kg. However a PNEC for trichloroacetic acid has been derived using longer-term data on plants and earthworms, of 2.4 μg/kg dry weight. A PNEC for monochloroacetic acid has also been derived from tests on plants as 6 μg/kg. As above, assuming that the toxicity of dichloroacetic acid will lie between that of the other two substances gives a PNEC of 4.2 μg/kg (mean of the two values. On a molar basis the mean value would be 5 μg/kg.

Risk characterisation

The conclusions here refer only to the risk from dichloroacetic acid produced from the breakdown of trichloroethylene in the atmosphere.

Aquatic compartment

The aerial deposition of dichloroacetic acid would be expected to contribute a disperse release to surface water. There are no measurements of concentrations in surface waters. The PNEC estimated is 0.72 μg/l. All the values measured in rainfall in south-western Germany were below this value, as were the mean values measured in Finland. Levels higher than this have been measured in Zürich, but do not take any dilution into account. The levels of dichloroacetic acid appear to decrease rapidly away from assumed anthropogenic sources of trichloroethylene, reaching levels close to the background within mainland Europe. It therefore appears that dichloroacetic acid derived from trichloroethylene will not have any widespread impact on the environment. It is possible that higher levels could be generated close to sites of emission of trichloroethylene. These would be diluted in surface water, and dichloroacetic acid is expected to degrade; therefore it seems unlikely that significant levels of dichloroacetic acid would persist from this source. However there are no surface water measurements to confirm this (any measured surface water levels would need to be considered carefully as there is clearly the potential for dichloroacetic acid production from chlorination of water).

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

Terrestrial compartment

There are limited data on levels of DCA in soil, with levels up to 0.85 μg/kg dry weight. Concentrations in soil from deposition of dichloroacetic acid have been estimated as 0.06 μg/kg or 0.024 μg/kg if leaching is included, which are lower than those measured. A PNEC for soil has been estimated from the aquatic value as 0.11 μg/kg, but a more realistic value of 4.2 μg/kg has been estimated from the PNECs for TCA and MCAA. Although limited these data would indicate no concern.

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Appendix C EUSES Output

The EUSES program has been used to calculate PEC values in the risk assessment. This appendix contains the output from the program. Some notes are needed to explain how the program was used.

Local emissions

There are six scenarios, as follows:

Scenario 1 - production (this is based on the largest actual site, but does not include site specific information and so the concentrations derived are not necessarily the same as those in the risk assessment report).

Scenario 2 - use as an intermediate (processing)

Scenario 3 - use in metal cleaning: formulation (corresponding to handling in the risk assessment) and processing (corresponding to use)

Scenario 4 - use in adhesives: formulation and processing (corresponding to use)

Scenario 5 - use in consumer products: formulation and private use (corresponding to use)

Scenario 6 - use in other products: processing (corresponding to use)

Regional

Releases to the regional and continental environments were calculated as indicated in the risk assessment report, and the total releases were entered directly into EUSES.

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

Appendix D Summary of ecotoxicity tests

Fish

Smith et al. (1991)

Acute and chronic tests on were carried out on American flagfish (*Jordanella floridae*). All tests used dechlorinated Lake Superior water taken from the Thunder Bay public supply, pH 6.95, alkalinity 44.9 mg/l as $CaCO_3$, hardness 48 mg/l as $CaCO_3$.

Acute tests

Static and flow through tests using the methods of "Committee on Methods for Toxicity Tests with Aquatic Organisms" (EPA-660/3-75-009). Temperature 25±2°C, 2-4 month old fish used. Acetone used as co-solvent to introduce chemicals into the diluter. Nominal acetone concentrations were 79-198 mg/l depending on diluter cell; measured concentrations of acetone in test tanks did not exceed 2% of LC_{50} for acetone (8,500 mg/l). Water and acetone (79-198 mg/l) controls were employed in both static and flow-through tests. No aeration used in either test but dissolved oxygen was >90% saturation in all tests.

Static tests - 3 litres of solution with 5 flagfish to each tank, 5 or 6 duplicate nominal concentrations in logarithmic series, solutions renewed every 24 hours. 96-hour LC_{50} 63.1 mg/l.

Flow-through tests - 30 litre aquaria, 10 fish per aquarium, flow rate 6 litres/hour, 5 or 6 duplicate concentrations in logarithmic series, each aquarium sampled at least 3 times to determine concentration. 96-hour LC_{50} 28.28 mg/l.

Chronic tests - similar to early life stage (ELS) test with fathead minnow. Used flow-through system similar to above, temperature 25±1°C, dissolved oxygen >90% saturation, tanks siphoned daily to remove bottom debris, concentrations analysed 5 days per week throughout 28-day exposure period. Two ages of fish used in simultaneous flow-through exposures:

- Embryo-larval fish test, with data collected on hatching success and 10-day larval survival. Began with 50 fertilised eggs (25 per duplicate) at each concentration and in the two controls; the eggs were less than 24 hours old. After hatching (4-6 days), transferred to fry retainers and held for a 10-day post-hatch exposure period.

- Week old fry test, with data generated on survival and growth over 28 days. 50 one week old fry per test level and controls (25 fry/duplicate). Growth measured by final weight.

MATC (geometric mean of NOEC and LOEC) values calculated for four endpoints, two for each test. For egg hatchability, MATC >21.2 mg/l; for 10-day larval survival, MATC = 11.0 mg/l. For 28-day fry survival, MATC = 14.85 mg/l; for 28 day fry growth, MATC >20.9 mg/l. Lowest NOEC was for 10-day larval survival, at 5.76 mg/l.

Acute flow through study and chronic studies considered valid.

Alexander et al. (1978)

Static and flow-through tests used. Adult fathead minnows (*Pimephales promelas*), ~1.04 g and 49 mm in length were used. Lake Huron water used, dechlorinated, pH 7.8-8.0. The methods of

"Committee on Methods for Toxicity Tests with Aquatic Organisms" (EPA-660/3-75-009) were used. Methanol or ethanol employed as carrier solvents, also used in controls. No concentration monitoring in static tests; aquaria were covered with plastic film for first 24 hours only; dissolved oxygen monitored and did not fall below 5.0 mg/l; ethanol used did not exceed 0.5 ml/l; 96-hour static LC_{50} 66.8 mg/l (nominal). In flow through test concentrations verified through monitoring; methanol used did not exceed 0.3 ml/l (note recommended maximum level is 0.1 ml/l); 96-hour flow through LC_{50} 40.7 mg/l (measured).

Although co-solvent exceeds recommended level, flow-through result considered valid.

Veith et al. (1983)

Flow through system using Lake Superior water at 25±1°C, hardness 45.5 mg/l as $CaCO_3$, alkalinity 42.2 mg/l as $CaCO_3$, pH 7.5, dissolved oxygen >60% of saturation. Fathead minnows (*Pimephales promelas*), 30 day old, weighing ~0.12 g. Control plus five different exposure concentrations, all in duplicate; concentrations measured daily. 96-hour LC_{50} = 44.1 mg/l.

Valid.

Slooff (1979)

Ten fish (*Brachydanio rerio*) exposed for 48 hours in 10 litre aquaria to each toxicant concentration. Flow rate in closed dynamic system was 6 litres/hour. Not clear how solutions were made up. LC_{50} determined as 60 mg/l. Although it is not clear how the solutions were made up this test is probably OK.

Use with care.

Ward et al. (1986)

Tests on marine fish *Cyprinodon variegatus*. Four to six day old fish exposed in 1.6 litre covered glass dish with 1 litre of solution. 10 fish per dish used; all treatments carried out in duplicate. Stock solution was made up as a 100% water soluble fraction (WSF) solution by adding 1 part trichloroethylene to 1,000 parts water (vol/vol) in covered flask and stirring for 1 hour, allowing to settle for 0.5-1 hour and then siphoning off the WSF solution. Test solutions were made up as 6.25, 12.5, 25, 50 and 100% of the WSF. Actual concentrations were measured at the start and end of the tests (or when 100% mortality occurred). The concentrations were found to decrease rapidly during the tests, by >75% over the first 24 hours and were less than 3% at 96 hours (but trichloroethylene was measurable in all except the lowest concentration solutions at the end of the exposures). Effect concentrations were calculated based on average measured concentrations as well as initial measured concentrations. 96-hour LC_{50} = 99 mg/l based on initial concentrations and 52 mg/l based on average concentration (average of initial and final).

Use with care - concentrations decreased significantly during test but were measured at beginning and end.

Slooff et al. (1983)

Short term tests on three fish species. Rainbow trout (*Oncorhynchus mykiss*) were 5-8 weeks old, tested at 15°C in tap water (pH 7-8); fathead minnows (*Pimephales promelas*) were 3-4 weeks old, tested at 20°C in Dutch standard water; and medaka (*Oryzias latipes*) were 4-5 weeks old,

tested at 24°C also in Dutch standard water. 10 fish in each test group. No other details provided. No concentration monitoring; effect concentrations estimated from nominal concentrations. 48-hour LC_{50} values were: *O mykiss* 42 mg/l; *O latipes* 270 mg/l; and *P promelas* 47 mg/l.

Use with care - nominal concentrations, no indication of precautions to prevent volatilisation.

Pearson and McConnell (1975)

Acute fish test in all glass flow-through apparatus; 5 dab (*Limanda limanda*, marine fish) used, 15-20 cm long; solutions not aerated, concentrations measured regularly. 96-hour LC_{50} = 16 mg/l.

Few details on study; use with care.

Könemann (1981)

Guppies (*Poecilia reticulata*), 2-3 months old, exposed in 1.5 litre vessels each with 1 litre of standard water (hardness 25 mg/l as $CaCO_3$) and covered with glass. Acetone or propan-2-ol stock solutions used to make up required concentration. Eight fish at each concentration; test solutions renewed daily; oxygen content > 5 mg/l; temperature 22±1°C. 7-day LC_{50} 54.8 mg/l.

Use with care - regular renewal but no concentration monitoring.

Loekle et al. (1983)

Long-term study to observe effects on *Poecilia sphenops* (black molly). Six adult fish (3 males, 3 females) exposed in 16 litre aquaria. Water was charcoal filtered, then aged and aerated for 10 days prior to test. Water in aquaria changed completely every two weeks. Fish exposed to two concentrations of trichloroethylene, 0.005 ml/l (7.3 mg/l) and 0.001 ml/l (1.5 mg/l) with one control, exposure period 60 days. Fish were removed daily if dead or severely distressed. All fish survived in control; five fish in each exposure group were removed. Test group fish showed reduced growth.

Use with care - long time periods between solution renewal, no concentration monitoring, endpoint not clearly defined.

Buccafusco et al. (1981)

Used "Methods for acute toxicity tests, EPA, 1975". Fish used were *Lepomis macrochirus*, 0.32-1.2 g each. Exposure vessels were 19.6 litres, containing 15 litres of solution and capped; 10 fish per vessel. Temperature 22±1°C. Water used was reconstituted deionised water according to EPA: hardness 32-48 mg/l as $CaCO_3$, alkalinity 28-34 mg/l as $CaCO_3$, pH 6.7-7.8, dissolved oxygen 7.0-8.8 mg/l. Several methods were used to make up stock solutions, including the use of co-solvents. It is not clear what method was used for trichloroethylene (a range of substances was tested) but the report states that undissolved chemical was present. Dissolved oxygen levels for many chemicals ranged from 9.7 mg/l at the start to 0.3 mg/l at the end of 96 hours, but it is not clear to which substances this applies. 96-hour LC_{50} = 45 mg/l (nominal).

Not valid; too many uncertainties over the exposure conditions.

Yoshioka et al. (1986)

Static exposures of *Oryzias latipes* (red killifish), 10 fish in 2 litres of test solution. Exposure at 20°C for 48 hours.

Not valid - nominal concentrations, no details of solution make-up, relatively short duration.

Juhnke and Ludemann (1978)

Few details, probably static exposure, nominal concentrations. Two laboratories, two results: LC_{50} = 136 and 203 mg/l.

Not enough information to judge validity.

Korte and Greim (1981)

Static exposure of *Brachydanio rerio* with containers covered; 48-hour exposure.

Not enough information to judge validity but apparently no renewal of solutions or concentration monitoring so not likely to be valid.

Invertebrates

Hermens et al. (1984)

48-hour tests on *Daphnia magna*, <2 days old. All tests carried out in duplicate with 25 organisms per exposure group in sealed vessels. Temperature 22±1°C, Dutch standard water used. Concentrations checked with GC; concentrations at beginning of tests were >70% of nominal, and decreased by <20% during tests. 48-hour IC_{50} = 2.20 μmol/litre, or 20.8 mg/l.

Test result considered valid.

Canton and Adema (1978)

Two laboratories carried out tests according to methods of Dutch Standardisation Institute (method reported in Adema, Hydrobiologia, 59(2), 125-134, 1978). Three organisms tested - *Daphnia magna* (1 day old), *D pulex* (1 day old) and *D cucullata* (11±1 day old in view of required size). Actual exposure concentrations not measured but exposure vessels sealed. 48-hour EC_{50}s were: for *D magna* 97 mg/l (lab I), 42 mg/l (lab II experiment 1) and 56 mg/l (lab II experiment 2); for *D pulex* 39-51 mg/l; and for *D cucullata* 56-58 mg/l.

Use with care, nominal concentrations.

Ward et al. (1986)

Tests on marine invertebrate *Mysidopsis bahia*. Three-day old organisms exposed in 1.6 litre covered glass dish with 1 litre of solution. 10 organisms per dish used; all treatments carried out in duplicate. Stock solution was made up as a 100% water soluble fraction (WSF) solution by adding 1 part trichloroethylene to 1,000 parts water (vol/vol) in covered flask and stirring for 1 hour, allowing to settle for 0.5-1 hour and then siphoning off the WSF solution. Test solutions were made up as 6.25, 12.5, 25, 50 and 100% of the WSF. Actual concentrations were measured at the start and end of the tests (or when 100% mortality occurred). The concentrations were found to decrease rapidly during the tests, by >75% over the first 24 hours and were less than 3% at 96 hours (but trichloroethylene was measurable in all solutions at the end of the exposures). Effect concentrations were calculated based on average measured concentrations as well as initial measured concentrations. 96-hour LC_{50} = 27 mg/l based on initial concentrations and 14 mg/l based on average concentration (average of initial and final).

Use with care - concentrations decreased significantly during test but were measured at beginning and end.

Bazin et al. (1987)

Method used was ISO 6341/AFNOR T-90-301. Concentration in stock solution checked by GC after making up, determined as 2,074 mg/l. Actual exposure solutions not analysed, sealed vessels used. Solutions made up in reconstituted hard water, pH 7.8-8.2, hardness 200 mg/l. Organisms (*Daphnia magna*) were less than 72 hours old. Few other details provided. 24-hour EC_{50} = 76 mg/l (3 tests, coefficient of variation 1%).

Use with care - few details, exposure concentrations not measured, but sealed vessels used and little variation between repeats.

LeBlanc (1980)

Daphnia magna, <24 hours old, tested in reconstituted water with total hardness 173±13 mg/l as $CaCO_3$ and pH 8±0.2, dissolved oxygen >60% saturation, temperature 22±1°C. Test carried out according to EPA method. Range of chemicals tested individually; 5-8 nominal concentrations of each chemical used. If the chemical was soluble then each concentration was tested in triplicate with 5 daphnids per solution, within 30 minutes of making up the solution. If the substance was volatile, the solution was not split into 3, hence exposure was probable 15 daphnids per concentration, but this is not clear from the paper. For trichloroethylene the exposure vessel was covered with a plastic wrap. 24-hour EC_{50} = 22 mg/l, 48-hour EC_{50} = 18 mg/l.

Use with care, nominal concentrations.

Korte and Greim (1981)

Acute test on *Daphnia magna* according to OECD Guideline of March 1979. No further details of the test given except that 25 ml glass flasks and ground glass stoppers were used. Dissolved oxygen levels were measured at the beginning and end of the tests, and were all >95% saturation. The EC_{50} derived was 27 mg/l.

Use with care - concentrations not measured, but precautions taken against volatilisation.

Abernethy et al. (1986)

Used the method described by Bobra et al (1983). Stock solution made up as a saturated solution in water. Test solutions made up by dilution from stock, and nominal concentration values calculated as fractions of solubility. (Note that concentration of trichloroethylene in "saturated" solutions may vary; value used here was 8,370 mmol/m^3, which is equivalent to 1.11 g/l, but other values of 2 g/l and 2.85 g/l have been obtained by similar methods). Tests carried out in 33 ml glass vials, with air spaces eliminated and teflon-lined screw caps. Initial dissolved oxygen concentration was 8-9 mg/l, and the lowest value after 48 hours was 5 mg/l. *Daphnia magna* of 4-6 days old were used. Concentrations in exposure solutions not measured. 48-hour EC_{50} 7.8 mg/l.

Test considered not valid - uncertainty over stock solution concentration and hence exposure concentrations, no concentration monitoring.

McCarty (1979)

Stock solution prepared in methanol at a concentration of 50 mg/ml. Test solutions made up with dechlorinated Lake Huron water: dissolved oxygen 7.7 mg/l, alkalinity 85 mg/l as $CaCO_3$, hardness 100 mg/l as $CaCO_3$, pH 7.6.EPA method used as in EPA 600/3-75-009, static test using first instar daphnids at 20±1°C. Exposures carried out in 250 ml beakers with 200 ml solution in each. Two controls run, one with dechlorinated Lake Huron water, the other with the largest amount of methanol present in test solutions (396 mg/l - note that maximum recommended is 100 mg/l). Ten daphnids added to each beaker, three beakers used for each concentration and for each control. Five tests run; first four considered invalid as there was more than 10% mortality in the controls (not clear which controls). No information given on what changes (if any) made to make the fifth test valid. Results showed an erratic dose-response curve. 48-hour LC_{50} presented as 2.2 mg/l.

Test considered not valid - no concentration monitoring, open containers, unexplained problems in controls, erratic dose-response.

Schuebel (1984)

The EG "Prolonged toxicity for *Daphnia magna*" No 79/831 Rev 1 test method was used. Daphnia were bred in dechlorinated drinking water at 20°C. The water was changed daily; the Daphnia were fed daily with green algae and with an additional 1% of specific food developed for breeding Daphnia. The same feed was used during the tests. For breeding the young animals were treated with streptomycin against mycobacterium. During the test a light:dark rhythm of 16:8 hours was used with a light intensity of ~600 lux. A stock solution of trichloroethylene of 150 mg/l was used. The tests were carried out in 350 ml Erlenmeyer flasks with ground glass stoppers to prevent volatilisation. Controls and four parallel test concentrations were used. The tests were carried out at 20.5-21.2°C.

It was found that a 21-day test was not possible unless the Daphnia had been treated with streptomycin. Use of the food supplement could also cause problems as it did not dissolve fully. Effects on growth were seen at 8 mg/l, and the NOEC was determined as 2.3 mg/l.

It is not entirely clear from the report whether the problems described affected the outcome of the tests with trichloroethylene (the study was aimed at developing the protocol). It is also not clear how frequently the solutions were changed during the test, nor what volume of solution was used within the 250 ml flasks. There was no concentration monitoring, and no indication of whether the pH or dissolved oxygen concentration was measured during the tests.

Use with care - for reasons given above, result not used in the derivation of the PNEC.

Kördel et al. (1984)

21-day test for effects on reproduction and mortality with *Daphnia magna*. The authors point out that the test did not fulfil the quality criteria. The concentrations of trichloroethylene fell below 80% of nominal within 48 hours, and there were possible problems with infection of the *Daphnia*. An NOEC of 0.15 mg/l was derived from the nominal concentrations.

Not valid.

Yoshioka et al. (1986)

Moina macropoda exposed to series of chemicals in medium. Ten organisms in 100 ml medium into 250 ml vessel, for 3 hours. Number of surviving organisms counted. LC_{50} for trichloroethylene determined as 200 mg/l.

Not valid - very short term exposure (although this makes the lack of concentration measurement less important).

US EPA (1980)

Original reference not seen.

Not possible to validate this study.

Microorganisms and Algae

Blum and Speece (1991a)

Tests on three types of bacteria: aerobic heterotrophs, *Nitrosomonas* and methanogens. Sealed 125 ml serum bottles used to prevent loss of volatile chemicals. A range of exposure concentrations was used, together with uninhibited controls and blanks containing no bacteria. Exposures carried out at least in duplicate, in some cases with 3 or 4 repeats. Bacteria were obtained from enrichment cultures and maintained in the test laboratory.

Aerobic heterotrophs were tested at 35°C and pH7, for up to 49 hours with shaking; test volume 50 ml; endpoint was inhibition of oxygen consumption. 24-hour IC_{50} determined as 130 mg/l.

Nitrosomonas tested at 25°C at pH 6.5-8.0 for 24 hours with shaking, test volume 50 ml. 24-hour IC_{50} determined as 0.81 mg/l. The authors considered that results less than 1.5 μmol/l for *Nitrosomonas* were questionable; this applies to trichloroethylene.

Methanogens were tested at 35°C at pH 7 for 24 hours, test volume 50 ml. Endpoint was gas production. 24-hour IC_{50} determined as 13 mg/l.

Performance of the control cultures was not reported. Concentrations after exposure were not measured, but exposure durations were short.

The results for aerobic heterotrophs and methanogens are considered valid. The *Nitrosomonas* result is not considered valid.

Volskay and Grady (1986)

Modified version of OECD 209 “Activated sludge respiration inhibition test” used. Original version not appropriate for volatile chemicals because continuous aeration is used. Modified method used more dilute cell and substrate concentrations, and conducted tests in vessels sealed with PTFE plug; headspace was eliminated and measurements of oxygen uptake rate were made without having to transfer contents to another container. Dissolved oxygen monitored through the test, and re-aeration took place when level dropped below 40% of saturation. Parallel control reactor (with water instead of test substance) used; measurements were made of oxygen concentrations over 5 minute periods separated by 20 minutes of stirring, and uptake in test reactor expressed as percentage of uptake in control reactor over same time. Temperature and pH

measured at end of test and required to be within limits to be valid (pH 6.7-7.3, temperature of reactors within 2°C of each other).

Substances tested at solubility limit or 1,000 mg/l; if inhibition was >50% then a range of concentrations was tested to derive an EC_{50}. 30 minute value for trichloroethylene was 260 mg/l.

Valid.

Brack and Rottler (1994)

Cell multiplication test with *Chlamydomonas reinhardii* (algae). Inocula preapred from 7-day old precultures; cell density at start of exposure $5 \cdot 10^3$ cells/ml. Tests conducted in sealed vessels, with carbon dioxide atmosphere provided by $K_2CO_3/KHCO_3$ buffer to give carbon dioxide concnetration of 1.14%. Concentration of trichloroethylene measured in exposure solutions by ECD. Biomass measured by extracting total chlorophyll, and analysing with fluorimetry. 72-hour EC_{50} 36.5 mg/l; 72-hour EC_{10}12.3 mg/l.

Valid.

Scheubel (1984)

A cell multiplication inhibition test for green algae carried out according to a UBA method. In repeated tests with different closures for the test vessels very different effect concentrations were found. The study concluded that the test guideline for inhibition of cell multiplication in green algae could not be applied to readily volatile chemicals since a gas-tight closure for the test vessel is not permitted. Results reported for 96-hour inhibition (in vessels with gas-tight stoppers): EC_{10} 43-61 mg/l.

An assimilation test was also carried out, a three generation growth test lasting 24 hours (DIN 38412 part 12/draft). The algae *Scenedesmus subspicatus* was used; six parallel tests carried out, three in the light and three in the dark. 100 ml flasks were used, filled with test or control cultures without headspace and closed with gas-tight stoppers. Tests carried out at 20°C for 24 hours; oxygen production measured. The EC_{10} value was determined as 82 or 70 mg/l (two different transformations of the data).

Use with care - precautions taken to prevent volatile loss of substance, but non-standard endpoint.

Blum and Speece (1991b)

This paper includes the results from Blum and Speece (1991b). It also includes a Microtox test (with *Photobacterium phosphoreum*, now called *Vibrio fisheri*). This was carried out at 15°C and pH 6.5-7.5, for 5 minutes in 1 ml open cuvettes. The IC_{50} value derived for inhibition of phosphorescence was 960 mg/l.

Valid but not relevant for PNEC for microorganisms.

DeZwart and Slooff (1983)

Microtox test with *Photobacterium phosphoreum* (now called *Vibrio fischerii*) according to procedure recommended by manufacturer. Test carried out at 15°C, for 5 or 15 minutes. The EC_{50} values derived for inhibition of phosphorescence were 156 and 164 mg/l (5 minutes) and

115 and 118 mg/l (15 minutes); the EC_{10} values were 87 mg/l (5 minutes) and 75 mg/l (15 minutes).

Valid but not relevant for PNEC for microorganisms.

Bazin et al. (1987)

Microtox test with *Photobacterium phosphoreum* (now called *Vibrio fischerii*), carried out at 15°C for 10 minutes. Average EC_{50} for 10 minutes was 602 mg/l (mean of 5 repeats).

Valid but not relevant for PNEC for microorganisms.

Hermens et al. (1985a)

Microtox test with *Photoacterium phosphoreum*. Test carried out according to procedures in manufacturer's manual. Five concentrations tested, fifteen minute exposures at 15°C. EC_{50} was 260 mg/l.

Valid but not relevant for PNEC for microorganisms.

Krebs (1985)

First test on assimilation - alteration of photosynthesis capacity (measured by oxygen production) on exposure to test substance. Used mixed culture of green algae (principal constituent *Scenedesmus*), at room temperature (20°C) and daylight conditions. Duplicates of 6 concentrations (one kept in dark, other in light) and 4 controls used. Test duration 24 hours; oxygen production calculated by difference between light and dark flasks, and related to production in controls. For trichloroethylene, EC_{10} = 230 mg/l and EC_{50} = 530 mg/l.

Second test looked at effects on bacterial degradation of nutrient medium (through oxygen consumption). Heterotrophic bacteria used, exposed for 24 hours at 20°C. For trichloroethylene, EC_{10} = 63 mg/l and EC_{50} = 160 mg/l.

Performance of controls not reported. No indication of measurement of concentrations during exposures. No indication of exposure vessels being sealed (but relatively short exposures). Only one replicate per concentration.

Use with care.

Bringmann and Kühn (1980a)

Toxicity thresholds for *Pseudomonas putida* (bacteria), *Scenedesmus quadricauda* (green algae) and *Entosiphon sulcatum* (protozoan) were determined. Pure cultures of single-cell organisms used and cell multiplication inhibition determined.

- *Pseudomonas putida* - concentration of the bacterial suspension measured turbidimetrically, threshold concentration taken as that where the extinction value drops more than 3% below the mean value in non-toxic solutions. Solutions neutralised; 4 parallel dilution series prepared in Erlenmeyer flasks stoppered with cotton-lined plastic caps, 3 series were inoculated with bacterial suspension, the fourth with saline as a blank series. Incubation was for 16 hours at 25°C. Toxicity threshold determined as 65 mg/l.

- *Scenedesmus quadricauda* - similar to *P putida*, but two dilution series made up. One series inoculated with algal suspension, the other had doubly distilled water added. 10 ml taken from each flask of inoculated dilution series into 3 Kapsenberg culture tubes, and 10 ml from non-inoculated series into 1 tube. Tubes stoppered with metal caps and kept at 27°C, 50% RH for 7 days. Toxicity threshold determined as >1,000 mg/l.

- *Entosiphon sulcatum* - again similar to *P putida*, but number of protozoa determined by cell counter. Two parallel dilution series in 300 ml Erlenmeyer flasks stoppered with metal caps, incubated at 25°C for 72 hours. Toxicity threshold determined as 1,200 mg/l.

No indication of the performance of the organisms in the controls. No indication that the concentrations of trichloroethylene were measured in the tests, but the vessels were sealed. No reported values for dissolved oxygen concentrations. Short exposures for *P putida* may not be affected too much by these, but longer exposures are more uncertain. Algal growth over 7 days is likely to have gone beyond the exponential growth phase.

Use with care for *P putida* result, others not considered valid.

Trénel (1990)

Bacteria test using methods of Bringmann (Bringmann and Kühn, 1977). Ring test of 11 laboratories. Mean toxic threshold after 16 hours 81 mg/l. No concentration monitoring. Details not seen, referenced in BUA (1994), original work from 1972.

Use with care - vessels likely to be open, no monitoring, but relatively short duration.

Robra (1979)

Reported the results of a ring test of 7 laboratories, looking at the inhibition of oxygen consumption in pure cultures of *Pseudomonas putida*. Bacteria used in exponential growth phase; tested at 20°C in 15 ml of solution. Average toxicity threshold concentration was 547 mg/l.

Use with care - few details reported.

Slooff et al. (1983)

Test on *Selenastrum capricornutum*; 96-hour test with algae in the log growth phase, initial cell density $5 \cdot 10^4$ cells/ml, 26°C. No monitoring of concentration. No other details available. EC_{50} based on nominal concentrations was 175 mg/l.

Use with care - few details reported, nominal concentrations.

Geyer et al. (1985)

Scenedesmus subspicatus tested according to guideline of the Umweltbundesamt. Stock cultures were prepared every 10 days in 20 ml nutrient solution, and exposed to constant lighting. Experiments carried out at 22±2°C. Test solutions made up with defined quantity of chemical diluted in defined quantity of sterilised doubly distilled water. Acetone used as carrier solvent up to maximum concentration of 0.1 ml in 1,000 ml, and the same amount of acetone added to the controls. Two parallel dilution series and four controls prepared in 300 ml Erlenmeyer flasks with Kapsenberg caps. Initial cell density ~10^4 cells/ml. After 0, 72 and 96 hours the cell growth

of a 10 mm layer of cell suspension from each test culture and the controls was measured at 587 nm. Concentration-effect curve plotted. Derived effect concentrations were 96-hour EC_{50} 450 mg/l, EC_{10} 300 mg/l.

Use with care - no indication that concentrations monitored.

Bringmann and Kühn (1978)

Toxicity threshold determined for blue-green algae (*Microcystis aeruginosa*) and green algae (*Scenedesmus quadricauda*) using cell multiplication inhibition test. Primary cultures prepared in 100 ml Erlenmeyer flasks closed with metal caps and kept at 27°C and 50% RH; new cultures prepared every 10 days. Two parallel dilution series prepared in 300 ml Erlenmeyer flasks, using a constant dilution ratio. Algal growth monitored by measuring the extinction of a 10 mm thick algal suspension from each flask at 578 nm. Exposures conducted for 7 days. For *M aeruginosa* the toxicity threshold was determined as 63 mg/l; for *S quadricauda* the threshold was >1,000 mg/l.

Use with care, nominal concentrations.

Yoshioka et al. (1985)

Tetrahymena pyriformis exposed in medium at 30°C for 24 hours in static test. Cell counts made and 50% inhibition concentration determined. Sealed vessels used for volatile chemicals (which chemicals these were was not specified). Vessels had headspace. Exposure concentrations not measured. EC_{50} determined as 410 mg/l.

Use with care - vessels sealed but headspace present and elevated temperature, nominal concentrations

Bringmann and Kühn (1980b)

Cell multiplication test with protozoan *Uronema parduczi*. Trichloroethylene solutions made up in standard mineral test medium, in two parallel dilution series, in 300 ml Erlenmeyer flasks with metal caps. Test cultures were fed with inactivated bacteria. Incubation was at 25°C, pH 6.9 for 20 hours. Cell counter used to quantify growth of cultures. Toxicity threshold determined as >960 mg/l.

No indication of concentration monitoring but the vessels were sealed; no information on performance of control cultures.

Not valid.

Bringmann and Kühn (1981)

Toxicity thresholds determined using analogous procedures to those above. Some results repeated from earlier studies. One further result, for *Chilomonas paramaecium Ehrenberg*, with a toxicity threshold of >400 mg/l.

Not considered valid for same reasons as above.

Ward et al. (1986)

Tests on *Skeletonema costatum* in synthetic seawater enriched with nutrients, at 20±1°C. Tested in 125 ml flasks containing 50 ml of test solution or control water. Each flask was inoculated with ~$2 \cdot 10^4$ cells/ml. Test concentrations and controls in triplicate. Stock solution was made up as a 100% water soluble fraction (WSF) solution by adding 1 part trichloroethylene to 1,000 parts water (vol/vol) in covered flask and stirring for 1 hour, allowing to settle for 0.5-1 hour and then siphoning off the WSF solution. Test solutions were made up as 6.25, 12.5, 25, 50 and 100% of the WSF. *In vivo* chlorophyll a measured after 24, 48, 72 and 96 hours exposure; cell counts made after 96 hours. Concentrations of trichloroethylene determined analytically at initiation and termination of exposures. However, no trichloroethylene was detected at the end of the tests. As concentration monitoring was not continuous, it is not possible to say when the concentrations dropped below the detection limit and so the actual exposures in this study are not known. EC_{50} based on nominal concentrations was 150 mg/l.

Not valid - actual exposure concentrations not known.

Pearson and McConnell (1975)

Toxicity of trichloroethylene to *Phaeodactylum tricornutum* assessed by measuring changes in uptake of carbon from atmospheric carbon dioxide during photosynthesis. Uptake of carbon dioxide measured using C^{14}-labelled sodium carbonate. EC_{50} determined as 8 mg/l. No further details.

Not enough information to assess validity.

Wackett and Householder (1989)

Cytotoxic effects of trichloroethylene on *Pseudomonas putida* F1 investigated through inhibition of cell growth. Cultures grown in mineral medium in Erlenmeyer flasks, inoculum supplied from culture containing toluene and grown to late exponential phase. Growth rate of *P putida* markedly inhibited by addition of trichloroethylene vapours - cell doubling time increased from 1.5 hours in controls to 5 hours in exposed flask.

No indication of level of exposure; exposure through air not relevant to assessment endpoint.

Not valid.

Appendix E Quantitative risk assessment for trichloroethylene

Note: Trichloroethylene was discussed in the Meeting of the Commission Group of Specialised Experts in the fields of Carcinogenicity, Mutagenicity and Reprotoxicity in Brussels 30-31 March 2000. A clear majority of the Specialised Experts agreed that classification of trichloroethylene in Category 2 for carcinogenicity is warranted, based on evidence in one animal species, namely tumours in the rat kidney, supported by epidemiological data showing an association between exposure and kidney tumours and non-Hodgkin's lymphoma in humans.

At the TM4 meeting 2000 it was asked that quantitative risk assessments should be performed on the basis of the T25 method in relation to non-Hodgkin's lymphoma. As indicated below lymphomas have been found in an inhalation study with NMRI female mice. This study has been used in the calculation. For comparison the T25 method has also been applied for inhalation induced kidney tumours. It should be noted that risk assessments should normally be based animal tumours that is used in the classification.

CA Name: 1,1,2-Trichloroethene
CAS No: 79-01-6
Index No: 602-027-00-9
EU classification: Carcinogenicity Category 2, mutagenicity Category 3
Conversion: 1 ppm = 5.47 mg/m^3

$ClCH=CCl_2$

C_2HCl_3 Mol.weight: 131.4

Exposure levels and route of exposure

Human exposure for trichloroethylene according to the Risk Assessment of Trichloroethylene (November 2000).

Workers*

Manufacture and recycling

Inhalation 11 mg/kg/d (10 ppm · [5.47 · 13.9/70]), dermal 1 mg/kg/d Total 12 mg/kg/d

Metal cleaning

Inhalation 54 mg/kg/d (50 ppm), dermal 30 mg/kg/d Total 84 mg/kg/d

Adhesive manuf. LEV used

Inhalation 22 mg/kg/d (20 ppm), dermal 30 mg/kg/d Total 52 mg/kg/d

Adhesive manuf LEV not used

Inhalation 152 mg/kg/d (140 ppm), dermal 30 mg/kg/d Total 182 mg/kg/d

Manufacture of HCFC133a and HFC 134

Inhalation 12 mg/kg/d (11.5 ppm), dermal 1 mg/kg/d Total 13 mg/kg/d

* "Light work" used in calculation. The inhalation volume for light work: 13.9 mg/m^3 (default)

Consumers

A total body burden of 62 mg/kg/d (27 mg/kg/d via inhalation and 35 mg/kg/d via dermal uptake) was estimated for the current use of trichloroethylene in consumer products (occasional use, exposure can be regarded as one-off acute events). However, according to classification, this use will no longer be acceptable.

Humans via environment

Environmental exposures of $1.5 \cdot 10^{-4}$ mg/kg/day for the regional model and 0.022 mg/kg/day for a local model (primarily inhalation: $1.3 \cdot 10^{-4}$ and 0.019 mg/kg/day, respectively).

Effective dose level in humans

The human kidney tumours of concern were primarily associated with high airborne concentrations of trichloroethylene in two studies from Germany (1,2) although an increase in the relative kidney cancer risk was also observed in a large retrospective cohort study of workers in aircraft maintenance in the USA (3).

The human non-Hodgkin's lymphoma studies are summarised in **Table E.1**. Only studies demonstrating an increased risk of non-Hodgkin's lymphoma are included. It is noted that the increases were non-significant in all the studies included. Little information is available concerning exposure and the epidemiological data are not suitable for quantitative risk assessment.

Table E.1 Data on human non-Hodgkin's lymphoma after trichloroethylene exposure

Study	Exposure	NON-HODGKIN=S LYMPHOMA		
		SIR	95% CI	Obs
Axelson et al. (1994) (4) **1,421 men (22,447 person-years)** Mortality	< 110 mg/m^3	1.6	0.5-3.6	5
Anttila et al. (1995) (5) **3,089 persons (59,905 person-years)** Morbidity	<165 mg/m^3	1.8	0.8-3.6	8
Spirtas et al. (1991) (6) **7,282 persons** Mortality		1.3	0.7-2.1	14
Blair et al. (1998) (3) **7,204** Mortality		2.0	0.9-4.6	28
Boice et al. (1999) (7) **2,267 persons (66,183 person-years)** Mortality		1.2	0.7-2.0	14

Effective dose level in animals

Trichloroethylene has been studied in several long-term experiments with mice and rats. The studies will be briefly summarised below.

Mice NTP has published two gavage studies. The results of the first NTP study (8) clearly indicated that trichloroethylene induced a hepatocellular carcinoma response in mice. It is concluded from the second NTP study (9) that trichloroethylene was carcinogenic for both sexes of B6C3F1 mice, causing hepatocellular carcinomas in males and females. Harderian gland adenomas in male and female mice may have been associated with the administration of trichloroethylene.

Three studies with inhalation of trichloroethylene have been published. Trichloroethylene administered by inhalation exposure has induced lung tumours in female ICR mice (males not tested) (10), male Swiss mice, and female B6C3F1 mice (11,12); lymphomas in female NMRI mice (13), and hepatomas in male Swiss mice (11,12).

Rats NTP has published three gavage studies. The results of the first NTP study (8) showed no significant tumour increases. It is concluded from the second NTP study (9) that trichloroethylene was carcinogenic in F344 rats, inducing renal tubular adenocarcinomas. Peritoneal mesothelioma in male rats may have been associated with the administration of trichloroethylene. Trichloroethylene was not carcinogenic in F344 female rats. From the third NTP study (14) with male and female ACI, August, Marshall, and Osborne-Mendel rats, it is concluded that trichloroethylene administration caused renal cell cytomegaly and toxic nephropathy in both sexes of the four strains. However, these are considered to be inadequate studies of carcinogenic activity. The incidences of interstitial cell tumours of the testis were increased in Marshall rats.

Three inhalation studies with trichloroethylene have been published. Trichloroethylene administered by inhalation exposure has induced Leydig cell tumours and renal tubular adenocarcinomas in male Sprague Dawley rats (11,12). No tumours were found in female Sprague-Dawley rats or in Wistar rats (exposed for 36 months) (10-13).

Lymphomas in mice after inhalation

The induction of lymphomas in mice after inhalation are summarised in **Table E.2**.

Table E.2 Lymphomas in mice exposed to trichloroethylene by inhalation

Tumour	Control	Low dose	High dose
NMRI Henschler et al. 1980 (13) (>99.9% purity, 0.0015% triethanolamine)		100 ppm (547 mg/m^3)	500 ppm (2735 mg/m^3)
Females Lymphomas	9/29 (31%)	17/30 (57%) (p<0.001)	18/28 (64%) (p=0.01)
Males; no significant tumour increases			

Remarks on study:

species, strain:	mice, NMRI, females
route:	inhalation
tumour:	lymphomas
duration:	18 months, 6 hours/day, 5 days/week exposure, experiment terminated after 30 months
note:	no information concerning historical controls

Lowest dose with a significant increased tumour-incidence
Control: 9/29 (31%)
Low dose: 17/30 (57%)
net %: $(17 \cdot [100/30] - 9 \cdot [100/29])/(100 - 9 \cdot [100/29]) = 37.2\%$

Daily dose per mice during the exposure period
6 hours x inhalation volume · mg TRI/l · (5/7) (for 7 days a week)
6h · 2.2 l/h (def.) · (0.001 · 547 · 5/7) = 5.16 mg/mice/day.

(def.): In case bodyweights, feed consumption data etc. are not specified, the default data set is used.

Daily dose per kg bodyweight during the exposure period
bodyweight is not specified: 25 gram (def.)[1]
i.e. 1,000/25 · 5.16 = 206.4 mg TRI/kg bodyweight per day.

Dose at this incidence of lymphomas when administration started after 6 weeks and exposure is for 24 months
18/24 (exposure) · 30/24 (observation) · 206.4 mg TRI/kg bodyweight per day = 193.5 mg TRI/kg bodyweight per day

T25 after 24 months inhalation exposure
T25 = 25/37.2 · 193.5 mg/kg/day = 130 mg/kg/day.

T25 dose descriptor for lymphomas in mice is 130 mg TRI/kg bodyweight per day.
HT25 dose descriptor for human based on the mice study is: 130 mg/kg/d.

Kidney adenocarcinomas in rats after inhalation exposure

The induction of kidney adenocarcinomas in rats after inhalation are summarised in **Table E.3**.

Table E.3 Kidney tumours in rats exposed to trichloroethylene by inhalation

Study/Tumour	Control	Low dose	Middle dose	High dose
Inhalation *Sprague-Dawley* Maltoni et al. (1986); 1988 (11,12) (>99.9% purity)		**100 ppm** (547 mg/m^3)	**300 ppm** (1,641 mg/m^3)	**600 ppm** (3,282 mg/m^3)
Males Kidney: Renal tubular adenocarcinomas (trend, p=0.001)	0/135 (0%)	0/130 (0%)	0/130 (0%)	4/130 (3%) (p=0.06)

Remarks on study:

species, strain:	rat, Sprague-Dawley, males
route:	inhalation
tumour:	kidney adenocarcinomas
duration:	104 weeks, 7 hours/day, 5 days/week

Lowest dose with a significant increased tumour-incidence

Control: 0/135 (0%)
High dose: 4/130 (3%)
net %: 3%

Daily dose per rat during the exposure period

7 hours · inhalation volume x mg TRI/l · (5/7) (for 7 days a week)
7h · 20.5 l/h (def.) · (0.001 · 3,282 · 5/7) = 336.4 mg/rat/day.

(def.): In case bodyweights, feed consumption data etc. are not specified, the default data set is used.

Daily dose per kg bodyweight during the exposure period

bodyweight is not specified: 500 gram (def.)[1]
i.e. 1,000/500 · 336.4 = 672.8 mg TRI/kg bodyweight per day.

T25 after 24 months inhalation exposure

T25 = 25/3 · 672.8 mg/kg/day = 5,607 mg/kg/day.

T25 dose descriptor for kidney adenocarcinomas in rats is 5,607 mg TRI/kg bodyweight per day.
HT25 dose descriptor for human based on the rat study is: 5,607 mg/kg/d.

Elements that may influence the calculated lifetime cancer risks

Data-sets available: One data-set from mice available.

Epidemiological studies: Workers exposed to trichloroethylene had a relative risk of non-Hodgkin's lymphoma of up to 2.0. In the study of Axelson et al. (4) a SIR (Standardised Incidence Risk) of 1.6 was observed for workers. The exposure level was < 110 mg/m^3 for the majority of workers. A risk assessment based on a relative risk of 1.6 and an exposure level of 110 mg/m^3 may represent an underestimation of the risk since the exposure has probably been lower and the exposure time less than a "working-life". For "light work" an exposure concentration of 110 mg/m^3 will represent a daily dose of 21.8 mg/kg body weight (i.e., 13.9 m^3/working day · 110 mg/m^3 /70 kg body weight). The lifetime probability of eventually die from non-Hodgkin's lymphoma in Norway is 0.005 in the general population. Based on this study the increase in lifetime cancer risk associated with an exposure of 21.8 mg/kg bw per day under occupational conditions of exposure is calculated to be $[(1.6 \cdot 5 \cdot 10^{-3}) - 0.005] = 3 \cdot 10^{-3}$ assuming that the death rate from non-Hodgkin's lymphoma is the same in Norway and Sweden.

The lifetime increase in cancer risk for lymphomas under occupational conditions of exposure calculated from the animal data is ([21.8/2.8]/[130/0.25]), i.e., $15 \cdot 10^{-3}$. Since the estimate from the study by Axelson et al. (4), i.e., $3 \cdot 10^{-3}$ probably represents an underestimate as pointed out above, the study substantiates the estimates from the animal studies. The corresponding increase in risk for kidney cancer would be ([21.8/2.8]/[5509/0.25]) = $3.5 \cdot 10^{-4}$ or 43 times less than the risk of non-Hodgkin's lymphoma.

Dose-response relationships: ----

Site/species/strain/gender activity: Tumour at different sites in both male and females rats and mice.

Mechanistic relevance to humans: Evidence for kidney cancer and non-Hodgkin's lymphoma in humans.

Toxicokinetics: ---

Other elements: ---

Conlusion: The most relevant study in relation to the risk of non-Hodgkin's lymphoma in humans is a female mice inhalation study. The calculated risk from epidemiological studies substantiates the risk estimates from the mice study. Since the risk estimate is based on only one study and no data concerning historical controls are available, it follows that the uncertainties may be considerable.

Lifetime increased cancer risk levels

*Workers:**

HT25: 130 mg/kg bw/d
Exposure level: 12 mg/kg bw/d
Lifetime cancer risk level: ([12/2.8]/[130/0.25]) $\boldsymbol{8.2 \cdot 10^{-3}}$

*Calculation based on the lowest exposure scenario given. Thus, the lifetime risk of death caused by non-Hodgkin's lymphoma will be higher for the other groups of exposed trichloroethylene workers.

Consumers:

HT25: 130 mg/kg bw/d
Exposure level: 62 mg/kg bw/event, equivalent to 0.17-2 mg/kg bw/d assuming 1-12 events/year
Lifetime cancer risk level: ([0.17-2/1.25*]/[130/0.25]) $\boldsymbol{2.6 \cdot 10^{-4} – 3.1 \cdot 10^{-3}}$

* applying a correction factor of 75/60 to correct for exposure time (60 years instead of lifetime)

However, it is to be noted that in the absence of actual exposure data the exposure was based on modelled data. The modelled exposure estimate may be unrealistically high, as may be the assumptions for frequency of use. Besides, according to the classification, the current use of trichloroethylene in consumer products will no longer be acceptable.

Humans exposed via the environment

HT25: 130 mg/kg bw/d
Exposure level (regional model): $1.5 \cdot 10^{-4}$ mg/kg bw/d
Exposure level (local model): 0.022 mg/kg bw/d

Lifetime cancer risk level (regional model): ($1.5 \cdot 10^{-4}$ /[130/0.25]) $\boldsymbol{2.9 \cdot 10^{-7}}$
Lifetime cancer risk level (local model): (0.022 /[130/0.25]) $\boldsymbol{4 \cdot 10^{-5}}$

Comments: The most relevant study in relation to the risk of non-Hodgkin's lymphoma in humans is a female mice inhalation study. The calculated risk from epidemiological studies substantiates the risk estimates from the mice study. Since the risk estimate is based on only one study and no data concerning historical controls are available, it follows that the uncertainties may be considerable. On the other hand the role of dermal exposure has not been included in the quantitative risk assessment. The risk for kidney cancer is expected to be about 50 times less than the risk for non-Hodgkin's lymphoma.

Conclusion

Based on occupational conditions of exposure, it can be concluded that the occupational exposure scenarios are associated with additional cancer risks of $\geq 8.2 \times 10^{-3}$/lifetime, hence with a substantial cancer risk (conclusion (iiib))**.**

For the current use, consumer exposure is associated with an additional cancer risk of $2.6 \cdot 10^{-4}$ – $3.1 \cdot 10^{-3}$/ lifetime, hence with a substantial cancer risk (conclusion iiiB). However, it is to be noted that in the absence of actual exposure data the exposure was based on modelled data. The modelled exposure estimate may be unrealistically high, as may be the assumptions for frequency of use. Besides, according to the classification, the current use of trichloroethylene in consumer products will no longer be acceptable.

For humans via the environment the lifetime conditions of exposure are associated with additional cancer risks of $2.9 \cdot 10^{-7}$ and $4 \cdot 10^{-5}$/lifetime, hence with a residual cancer risk (conclusion (iiia)) (although borderline for the local model).

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European Commission

EUR 21057 EN **European Union Risk Assessment Report trichloroethylene, Volume 31**

Editors: B.G. Hansen, S.J. Munn, F. Berthault, C. Musset, S. Pakalin, M.Luotamo, J. de Bruijn, S. Vegro, G. Pellegrini, R. Allanou, S. Scheer.

2004 – X pp., 336 pp. – 17.0 x 24.0 cm

Environment and quality of life series

The report provides the comprehensive risk assessment of the substance trichloroethylene. It has been prepared by the UK 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 man 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 environmental risk assessment for trichloroethylene concludes that there is concern for plants exposed via the atmosphere as a consequence of exposure arising from production, formulation for solvent use and use in metal cleaning.

The human health risk assessment for trichloroethylene concludes that there is concern for workers, consumers and humans exposed via the environment as the substance is classified as a non-threshold carcinogen.

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European Union Risk Assessment Report

trichloroethylene

CAS No: 79-01-6 EINECS No: 201-167-4

Series: 1st Priority List Volume: 31