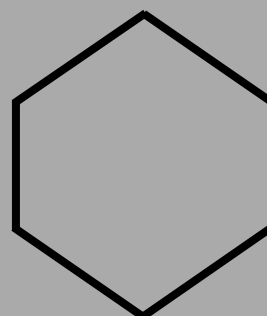


European Union Risk Assessment Report

CAS No: 110-82-7

EINECS No: 203-806-2

cyclohexane



1st Priority List

Volume: **41**

European Union Risk Assessment Report

CYCLOHEXANE

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RISK ASSESSMENT

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CYCLOHEXANE

CAS No: 110-82-7

EINECS No: 203-806-2

RISK ASSESSMENT

Final Report, 2004

The risk assessment of cyclohexane has been prepared by France on behalf of the European Union.

Contact points:

Environment: Ministère de l'aménagement du territoire et de l'environnement
DPPR/SDPD/BSPC
20, avenue de Ségur
75302 Paris 07 SP
Tél: + 33 1 42 19 15 42; Fax: + 33 1 42 19 14 68

The scientific work has been prepared by:

INERIS
Parc Technologique ALATA - BP n° 2
60550 Verneuil en Halatte
Tél: + 33 3 44 55 62 16; Fax: + 33 3 44 55 67 67

Human health: Ministère de l'emploi et de la solidarité
20 bis rue d'Estrées
75700 Paris
Tél: + 33 1 44 38 38 38; Fax: + 33 1 44 38 26 48

The scientific work has been prepared by:

Institut National de Recherche et de Sécurité (INRS)
Département Risques Chimiques et Biologiques
30, rue Olivier Noyer
75680 Paris cedex 14
Tél: + 33 1 40 44 30 83; Fax: + 33 1 40 44 30 54

Date of Last Literature Search:	1999
Review of report by MS Technical Experts finalised:	2001
Final report:	2004

Foreword

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

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93¹ 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.

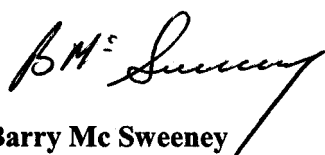
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each 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) 1488/94², which is supported by a technical guidance document³. 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
Director-General
DG Joint Research Centre



Catherine Day
Director-General
DG Environment

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]

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OVERALL RESULTS OF THE RISK ASSESSMENT

CAS-No.: 110-82-7
EINECS-No.: 203-806-2
IUPAC name: cyclohexane

Environment

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

This conclusion applies to all environmental compartments and all identified uses of cyclohexane: use as a chemical intermediate, use as a solvent in chemical production processes and use as a solvent in adhesives and coatings.

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 because of concerns for acute toxicity (neurobehavioural effects) and general systemic toxicity (hepatic effects) as a consequence of inhalation exposure arising from formulation and industrial use of products containing the substance as well as from use of products containing the substance in craft industries.

Consumers

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 because of concerns for acute toxicity (neurobehavioural effects) as a consequence of exposure arising from use of products containing the substance.

Humans exposed via the environment

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

Human health (risks from physicochemical properties)

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

CONTENTS

1	GENERAL SUBSTANCE INFORMATION	5
1.1	IDENTIFICATION OF THE SUBSTANCE	5
1.2	PURITY/IMPURITIES, ADDITIVES	5
1.3	PHYSICO-CHEMICAL PROPERTIES	5
1.4	CLASSIFICATION	6
2	GENERAL INFORMATION ON EXPOSURE	7
2.1	PRODUCTION	7
2.2	USE	7
2.3	EMISSION PATTERN	9
3	ENVIRONMENT	10
3.1	ENVIRONMENTAL EXPOSURE	10
3.1.1	Environmental fate	10
3.1.1.1	Degradation	10
3.1.1.2	Distribution	11
3.1.1.3	Accumulation	12
3.1.2	Aquatic compartment	13
3.1.2.1	Releases to surface water	13
3.1.2.1.1	Production	13
3.1.2.1.2	Use as an intermediate	13
3.1.2.1.3	Use as a solvent for production processes in chemical industry	15
3.1.2.1.4	Formulation and use of adhesives and coatings	19
3.1.2.1.5	Emissions not covered by the life-cycle of the produced/imported substance	20
3.1.2.2	Estimation of local aquatic concentrations	20
3.1.2.2.1	Production	20
3.1.2.2.2	Use as an intermediate	20
3.1.2.2.3	Use as a solvent for production processes in the chemical industry	21
3.1.2.2.4	Formulation and use of adhesives and coatings	22
3.1.2.3	Regional concentration in surface water	22
3.1.2.4	Monitoring data	23
3.1.2.5	Sediment	23
3.1.3	Atmosphere	24
3.1.3.1	Releases to atmosphere	24
3.1.3.1.1	Production	24
3.1.3.1.2	Use as an intermediate	25
3.1.3.1.3	Use as a solvent for production processes in the chemical industry	26
3.1.3.1.4	Formulation and use of adhesives and coatings	27
3.1.3.1.5	Emissions not covered by the life-cycle of the produced/imported substance	28
3.1.3.2	Estimation of local air concentrations and deposition rates	29
3.1.3.3	Local concentration due to car exhausts	29
3.1.3.4	Regional concentration in air	31
3.1.3.5	Monitoring in the atmosphere	31

3.1.4	Terrestrial compartment.....	32
3.1.4.1	Estimation of local soil and groundwater concentrations.....	32
3.1.4.2	Regional concentration in soil and groundwater.....	33
3.1.4.3	Monitoring data.....	33
3.1.5	Secondary poisoning.....	33
3.2	EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT.....	34
3.2.1	Aquatic compartment (incl. sediment).....	34
3.2.1.1	Toxicity test results.....	34
3.2.1.2	Comparison with (Q)SAR data.....	35
3.2.1.3	Determination of Predicted No Effect Concentration (PNEC).....	36
3.2.1.4	Sediment.....	36
3.2.2	Atmosphere.....	36
3.2.3	Terrestrial compartment.....	37
3.2.4	Secondary poisoning.....	37
3.3	RISK CHARACTERISATION.....	38
3.3.1	Aquatic compartment (incl. sediment).....	38
3.3.2	Atmosphere.....	38
3.3.3	Terrestrial compartment.....	39
3.3.4	Secondary poisoning.....	39
4	HUMAN HEALTH.....	40
4.1	HUMAN HEALTH (TOXICITY).....	40
4.1.1	Exposure assessment.....	40
4.1.1.1	Occupational exposure.....	40
4.1.1.1.1	Measured inhalation exposure data.....	40
4.1.1.1.2	Inhalation and dermal exposure data derived from modelling.....	45
4.1.1.1.1	Conclusion of occupational exposure.....	46
4.1.1.2	Consumer exposure.....	46
4.1.1.3	Humans exposed via the environment.....	47
4.1.1.4	Combined exposure.....	48
4.1.2	Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment.....	49
4.1.2.1	Toxicokinetics, metabolism and distribution.....	49
4.1.2.1.1	Oral.....	49
4.1.2.1.2	Dermal.....	50
4.1.2.1.3	Inhalation.....	51
4.1.2.1.4	Additional data: enzymatic induction.....	54
4.1.2.1.5	Summary of toxicokinetics, metabolism and distribution.....	54
4.1.2.2	Acute toxicity.....	55
4.1.2.2.1	Oral exposure.....	55
4.1.2.2.2	Dermal exposure.....	56
4.1.2.2.3	Inhalation exposure.....	56
4.1.2.2.4	Other information.....	61
4.1.2.2.5	Summary of acute toxicity (excluding acute neurotoxicity).....	61
4.1.2.2.6	Summary of acute neurotoxicity.....	61
4.1.2.3	Irritation.....	61
4.1.2.3.1	Studies in animals.....	61
4.1.2.3.2	Studies in humans.....	62
4.1.2.3.3	Summary of irritation.....	63
4.1.2.4	Corrosivity.....	63
4.1.2.5	Sensitisation.....	63
4.1.2.5.1	Skin sensitisation.....	63
4.1.2.5.2	Respiratory sensitisation.....	64
4.1.2.5.3	Summary of sensitisation.....	64

4.1.2.6	Repeated dose toxicity.....	64
4.1.2.6.1	Studies in animals.....	64
4.1.2.6.2	Studies in humans.....	69
4.1.2.6.3	Summary of repeated dose toxicity.....	70
4.1.2.7	Mutagenicity.....	72
4.1.2.7.1	<i>In vitro</i> studies.....	72
4.1.2.7.2	<i>In vivo</i> studies.....	74
4.1.2.7.3	Summary of mutagenicity.....	74
4.1.2.8	Carcinogenicity.....	74
4.1.2.9	Toxicity for reproduction.....	76
4.1.2.9.1	Two-generation reproduction studies (rat).....	76
4.1.2.9.2	Developmental toxicity studies.....	78
4.1.2.9.3	Summary of toxicity for reproduction.....	80
4.1.3	Risk characterisation.....	81
4.1.3.1	General aspects.....	81
4.1.3.2	Workers.....	81
4.1.3.3	Consumers.....	85
4.1.3.3	Humans exposed via the environment.....	86
4.1.3.4	Combined Exposure.....	86
4.2	HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES).....	87
4.2.1	Exposure assessment.....	87
4.2.1.1	Workers.....	87
4.2.1.2	Consumers.....	87
4.2.1.3	Humans exposed via the environment.....	87
4.2.2	Effects assessment: Hazard assessment.....	87
4.2.2.1	Explosivity.....	87
4.2.2.2	Flammability.....	87
4.2.2.3	Oxidising potential.....	88
4.2.3	Risk characterisation.....	88
5	RESULTS.....	89
5.1	ENVIRONMENT.....	89
5.2	HUMAN HEALTH.....	89
5.2.1	Human health (toxicity).....	89
5.2.1.1	Workers.....	89
5.2.1.2	Consumers.....	89
5.2.1.3	Humans exposed via the environment.....	89
5.2.2	Human health (risks from physico-chemical properties).....	89
6	REFERENCES.....	90
	ABBREVIATIONS.....	97
	Annex 1.....	103

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

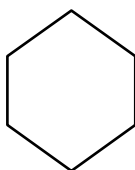
TABLES

Table 1.1	Summary of physico-chemical properties	5
Table 2.1	List of producers/importers having submitted a HEDSET diskette	7
Table 2.2	Use pattern	9
Table 3.1	Estimated biodegradation rate constants of cyclohexane	11
Table 3.2	Estimated water/solids partition coefficients in different compartments	11
Table 3.3	Estimated equilibrium distribution (fugacity model, level I)	12
Table 3.4	Estimated distribution in a STP (SIMPLETREAT)	12
Table 3.5	Specific data on releases from several producers	13
Table 3.6	Releases during use as an intermediate at several sites	14
Table 3.7	Estimations of releases due to industrial use of cyclohexane as solvent in chemical processes at several specific industrial sites	17
Table 3.8	Estimation of local aquatic concentrations at five specific production sites	20
Table 3.9	Estimation of local aquatic concentrations at several specific transformation sites	21
Table 3.10	Estimations of releases due to industrial use of cyclohexane as a solvent in chemical processes at several specific industrial sites	21
Table 3.11	Monitoring of cyclohexane in effluents or surface water	23
Table 3.12	Estimation of local releases at production sites in the EU	25
Table 3.13	Estimation of local releases at sites using cyclohexane as an intermediate in the EU	26
Table 3.14	Estimations of releases due to the industrial use of cyclohexane as solvent in chemical processes at several specific industrial sites	26
Table 3.15	Local concentrations in air during emissions, average air concentrations and average deposition rates	29
Table 3.16	Monitoring of cyclohexane in the atmosphere	31
Table 3.17	Local concentrations in soil and groundwater	32
Table 3.18	PEC/PNEC for all relevant exposure scenarios	38
Table 3.19	PEC/PNEC for all relevant exposure scenarios in soil	39
Table 4.1	Occupational Exposure Limits	40
Table 4.2	Measured 8-hour Time Weighted Average exposure data (ppm)	41
Table 4.3	Short term or task measurements (ppm)	41
Table 4.4	Summary of De Rosa et al. study on cyclohexane exposure	42
Table 4.5	Data from the Occupational Hygiene Visits Register (OHVR) database of the Health and Safety Executive	44
Table 4.6	Data from the COLCHIC database of INRS (France), 1995	44
Table 4.7	Conclusion of occupational exposure assessment	46
Table 4.8	Total daily intake due to local and regional environmental exposures	47
Table 4.9	Relationship between urinary solvent concentration and solvent exposure	52
Table 4.10	Summary of dependent variables of the two-choice visual discrimination task	58
Table 4.11	Blood and brain cyclohexane concentration following inhalation administration of 400–2,000 and 8,000 ppm in rats	59
Table 4.12	Summary of repeated dose toxicity studies	71
Table 4.13	<i>In vitro</i> genotoxicity data on cyclohexane	73
Table 4.14	Tumour promoting potential of cyclohexane according to the initiation-promotion protocol	75
Table 4.15	Mean body weights and mean body weight gains (g) of P1 female rats during gestation	77
Table 4.16	Mean body weights and mean body weight gains (g) of F1 female rats during gestation	77
Table 4.17	Mean pup numbers and survival : F1 generation	78
Table A1	Physiological, biochemical parameters and partition coefficients used in the PbPk model for cyclohexane	104

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS-No.: 110-82-7
EINECS-No.: 203-806-2
IUPAC name: cyclohexane
Synonyms: hexahydrobenzene, hexamethylene
Molecular weight: 84.16
Molecular formula: C₆H₁₂
Structural formula:



1.2 PURITY/IMPURITIES, ADDITIVES

Degree of purity of the produced/imported products within the EU: 99.8-99.9%

Impurities:

Linear and branched aliphatic hydrocarbons (Alkanes, specially n-hexane $\leq 0,02\%$)	< 0.1%
Alicyclic hydrocarbons (Cyclopentane and alkylcycloalkanes, especially methylcyclohexane:	< 0.12%
Benzene	0.005-0.06%)
Toluene	0.002-0.012%
	< 0.001%

Additives: none

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Summary of physico-chemical properties

Properties	Values	Reference
Physical state	liquid at 20°C and 101.3 kPa	
Melting point	6.5°C	CRC (1988); Merck (1989)
Boiling point	80.7°C	CRC (1988); Merck (1989)
Density	779-784 kg/m ³	Wintershall (1992)
Vapour pressure	10,300 Pa at 20°C	Wintershall (1992)
Surface tension ¹⁾	n.a.	IUPAC (1986)
Water solubility ²⁾	58 mg/l at 25°C	
n-octanol/water partition coefficient (log ₁₀)	3.44 measured value	Hansch and Leo (1985)

Table 1.1 continued overleaf

Table 1.1 continued Summary of physico-chemical properties

Properties	Values	Reference
Flash point	-20°C (closed cup)	Merck (1989)
Auto flammability	260°C	Merck (1989)
Flammability limit in air	1.3-8.4% (volume)	Merck (1989)

- 1) Due to its chemical structure (lack of polar radicals), cyclohexane is not expected to be surface active and no test was requested. A value of 25.5 dyne/cm is available for the pure liquid (CRC, 1988).
- 2) A review of all available data on water solubility was prepared by IUPAC (1986). A « best value » could only be derived for 25°C.

1.4 CLASSIFICATION

The classification and labelling of cyclohexane has recently been discussed (November 2000) and provisional agreement has been reached that the safety phrase S25 (“Avoid contact with eyes”) should be added to the current classification in Annex I of Directive 67/548/EEC. :

Classification F; R11

Xn; R65

Xi; R38

R67

N; R50-53

Labelling

F; Xn; N

R: 11-38-65-67-50/53

S: (2-)9-16-25-33-60-61-62

R 11: Highly flammable

R 65: Harmful: may cause lung damage if swallowed

R 38: Irritating to skin

R 67: Vapours may cause drowsiness and dizziness

R 50/53 Very toxic to aquatic organisms / may cause long-term adverse effects in the aquatic environment

S9: Keep container in a well-ventilated place

S 16: Keep away from sources of ignition – No smoking

S 25: Avoid contact with eyes

S 33: Take precautionary measures against static discharges

S 2: Keep out of the reach of children (only for consumer products)

S 60: This material and/or its container must be disposed of as hazardous waste

S 61: Avoid release to the environment. Refer to special instructions/safety data sheet

2

GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Data from 11 producers/importers are included in the IUCLID database. These are listed in **Table 2.1**.

Table 2.1 List of producers/importers having submitted a HEDSET diskette

BASF Antwerpen N.V., BE
Elf Atochem, F
Ertoil, ES
Exxon Chemical Holland, NL
N.V. Fina-Borealis, BE
ICI Chemicals & Polymers Ltd, UK
Kuwait Raffinatione e Chimica, IT
Proquimed S.A., ES
Rhone-Poulenc Chemicals Ltd, UK
Ruhr Oel GmbH, DE
Wintershall AG, DE

The total production volume in the EU is 835,000-925,000 t/a. The import volume is ca. 18,000-63,000 t/a. According to the Aromatics Producers Association (APA, 1995), the export volume is ca. 25,000 t/a. The total volume of cyclohexane used within the EU is therefore estimated at 828,000-963,000 t/a. Worldwide cyclohexane capacity exceeds 4,400,000 t/a as of January 1997 (SRI, 1997). For this assessment, it is assumed that a volume of 900,000 t/a are used in the EU.

Cyclohexane can either be produced by hydrogenation of benzene or by recovering naturally occurring cyclohexane from hydrocarbon streams. Typical temperatures and pressures during hydrogenation of benzene are 150 to 260°C and 2.1 to 3.5 MPa. Nickel, platinum or palladium is used as catalysts. Cyclohexane of low purity can be commercially obtained by conventional distillation of petroleum liquids. To obtain cyclohexane with a purity greater than 85%, it must be dehydrogenated to benzene, which can be further purified and dehydrogenated (US EPA, 1980).

Only two plants in the USA produce cyclohexane by distillation of petroleum liquids. In Western Europe and Japan, hydrogenation of benzene is used exclusively (SRI, 1997).

2.2 USE

Almost all the cyclohexane produced (96%) is used as a synthesis intermediate in the first step of nylon manufacture, both nylon 6 and nylon 66. Nylon 66 is based on combining two monomers-adipic acid and hexamethylenediamine (HMDA). Adipic acid is made by oxidising cyclohexane. HMDA is made mainly from butadiene and acrylonitrile, although a few companies make HMDA via adipic acid from cyclohexane. To make Nylon 6 requires only a

single monomer, caprolactam. Over 70% of the world caprolactam is made from cyclohexane, while the remaining 30% uses phenol or toluene as the starting material (Luger, 1996).

According to US EPA (1994a), 58% of the US-produced cyclohexane is used in the production of adipic acid.

Other uses are as a solvent in the chemical industry and as a component of products:

- cyclohexane is used as an auxiliary in chemical production processes, mainly as a precipitating and extraction agent, but also as a reaction enhancer.
- cyclohexane is used as a component of products in various industrial sectors (formulation and use of products), in craft industries (artisans) and in the public domain.

The main use as a solvent is in adhesives (cyclohexane combined with other solvents). These are mostly "neoprene" (polychloroprene) based adhesives used in the leather industry (shoes), the construction industry (floor coatings) and the automobile equipment industry. According to the French product register kept by INRS (Institut National de Recherche et de Sécurité, 1995) containing data provided by product formulators, the content of cyclohexane in these adhesives is most often between 10 and 30%.

Cyclohexane is also found in smaller quantities in styrene-butadiene-styrene, styrene-isoprene-styrene or natural rubber-based adhesives used in the manufacture of shoes and bedding equipment.

The information contained in the Danish product register confirms the above described profile. The typical cyclohexane content in adhesives in Denmark is 10-15% (Danish EPA, 1995).

A quick survey among French adhesives producers organised by the French federation of the paints, inks, colours and adhesives industry (FIPEC, 1995) among 16 adhesives producers (8 replies) revealed that ca. 1,300 t/a of cyclohexane are used by 8 companies at 11 sites for the formulation of polychloroprene (neoprene) adhesives.

In the Swedish Product Register for 1993 (personal communication from KEMI 1995), 46 products containing a total quantity of 114 tons cyclohexane are registered. The most frequent product types are solvents (90 t/a) and adhesives (10 t/a).

Other low quantity uses of cyclohexane are (no data on volumes for these uses are available so far):

- as a constituent of a solvent mixture in industrial coatings and polystyrene based paints,
- as a component in the interior coating of food packaging (this application is in development only),
- as an additive in printer inks,
- as an extraction solvent (pure or in association with other solvents) for pharmaceuticals or essential oils,
- as a separation/dilution agent in analytical chemistry,
- as an azeotropic agent for alcohol dehydration.

Cyclohexane is also used as a solvent in products (mainly adhesives) by craftsmen (shoe repairers, carpet layers, decorators).

In the general public field, cyclohexane is used in household and "do-it-yourself" products (adhesives, solvents) with possibly the same range of concentrations (10-30%).

For this assessment, an average content of cyclohexane in adhesives and coatings of 20% will be assumed.

Based on the aforementioned data, the following use pattern will be used for this assessment:

Table 2.2 Use pattern

Description	IC	UC	Quantity	Fraction
Intermediate in the chemical industry	3	33	864,000 t/a	96%
Solvent in chemical production processes	2	48	18,000 t/a	2%
Solvent in adhesives and coatings	14	48	18,000 t/a	2%

2.3 EMISSION PATTERN

Cyclohexane is produced in closed systems. As the production process does not involve water, the releases to wastewater should be low. Nevertheless, releases to water during production are possible e.g. through pumps or flue gas scrubbing. Atmospheric emissions are probable.

Processing related to the use as an intermediate for the production of nylon takes place in closed systems. The emissions into the environment are also mainly atmospheric.

High emissions are expected during the use of cyclohexane as a solvent.

Cyclohexane may also be released into the environment from the following sources:

- natural sources (crude oil, plants),
- combustion products (tobacco smoke, volcanic emissions),
- petroleum derived fuels (gasoline vapors).

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental fate

3.1.1.1 Degradation

Hydrolysis

No test on cyclohexane hydrolysis was carried out. Based on the molecular structure of cyclohexane, hydrolysis is not expected to be an important fate process.

Biodegradation

While linear alkanes and alkenes appear to be clearly readily biodegradable (CITI, 1992), conflicting results were found with cyclohexane.

Several test results on ready biodegradation are available:

- Manometric-Respirometry-Test (OECD GL 301 F): 77% degradation after 28 days, lag-time ca. 12 days (12-13 days in 2 of the replicates and 20 in the third replicate). The 10-day window criterion was fulfilled. The log-phase was very short in 2 of the 3 replicates, between 3 and 4 days, and approx. 7 days in the third replicate (Exxon, 1995).
- Manometric-Respirometry-Test (OECD GL 301 F): 6% degradation after 28 days (BASF, 1990).
- MITI-I-Test (OECD GL 301 C): 0.6% after 14 days (CITI, 1992).

As the test duration in the MITI test was only 14 days, the result is not conclusive, especially as a long lag-phase was observed in the respirometry test by Exxon (1995). Regarding the interpretation of the biodegradability potential of cyclohexane, two opposing results remain. The test by BASF (1990) was performed in 1989, at a time when the official OECD method had not yet been adopted. It is not clear whether significant deviations from the finally adopted method remained in the draft protocol.

According to Verschueren (1983), the first step of cyclohexane biodegradation is oxidation to cyclohexanol. Cyclohexanol can clearly be considered as readily biodegradable (CITI, 1992).

Furthermore, the possible biodegradation of cyclohexane has been proven in a non-standardised test. The test was performed with sterile saltwater inoculated with hydrocarbon oxidising bacteria. The inoculum concentration is not reported. The biodegradation rate of cyclohexane was 70% after 35 days, approximately the same biodegradation rate as for n-octane and n-hexadecane under the same conditions (Zobell, 1966).

Overall, it can be concluded that cyclohexane is readily biodegradable in the aquatic environment.

Test results from standard test systems for soil biodegradation are not available. In a non-standard test, no biodegradation was observed after 10 weeks (Haider et al., 1974). As the

test system was open and no mass balance was performed, no conclusion can be drawn from the result.

Therefore, as no results from biodegradation simulation tests in STPs, in surface water and soil are available, the degradation rates have to be estimated based on the "ready biodegradability" classification and the partition behaviour of cyclohexane according to the method described in the Technical Guidance Document (TGD) for risk assessment of existing chemicals (EC, 1996).

Table 3.1 Estimated biodegradation rate constants of cyclohexane

Compartment / medium	Biodegradation rate
Activated sludge (WWTP)	$k_{STP} = 1 \text{ h}^{-1}$
Surface water	$k_{sw} = 0.046 \text{ d}^{-1}$
Sediment	$k_{sed} = 0.0023 \text{ d}^{-1}$
Soil	$k_{soil} = 0.023 \text{ d}^{-1}$

1) The biodegradation rates in sediment and soil take account of adsorption to solid matter ($K_{oc} = 770 \text{ l/kg}$, see below).

Photooxidation

In the atmosphere, cyclohexane will react with the photochemically produced hydroxyl radicals. Based upon an atmospheric concentration of $5 \cdot 10^5 \text{ OH/cm}^3$, the atmospheric half-life of cyclohexane has been estimated to be 52 hours (Atkinson, 1985). This value is based on experimental results covering a temperature range of 23-224°C.

3.1.1.2 Distribution

The Henry's law constant can be estimated using the vapour pressure of 10,300 Pa and a water solubility of 58 mg/l: $H = 14,900 \text{ Pa} \cdot \text{m}^3/\text{mol}$ at 20°C. Cyclohexane is very volatile from water.

No test on adsorption and desorption of cyclohexane was performed. According to the TGD (EC, 1996), the K_{oc} can be calculated as $\log K_{oc} = 0.81 \cdot \log K_{ow} + 0.1$, based on results obtained for halogenated and non-halogenated hydrocarbons. With a $\log K_{ow}$ -value of 3.44, a K_{oc} -value of 770 l/kg is derived.

Based on the K_{oc} of 770 l/kg, the partition coefficients in the different compartments can be estimated using default organic carbon contents in the different compartments (**Table 3.2**).

Table 3.2 Estimated water/solids partition coefficients in different compartments

Compartment	OC content in solid phase	Solids-water partition coefficient	Total compartment/water partition coefficient
Soil-water	2%	$K_{p_{soil}} = 15.4 \text{ l/kg}$	$K_{soil_water} = 24.6 \text{ m}^3/\text{m}^3$
Sediment-water	5%	$K_{p_{sed}} = 38.5 \text{ l/kg}$	$K_{sed_water} = 20.1 \text{ m}^3/\text{m}^3$
Suspended matter-water	10%	$K_{p_{susp}} = 77 \text{ l/kg}$	$K_{susp_water} = 20.0 \text{ m}^3/\text{m}^3$

Using the fugacity model of Mackay (level 1), the theoretical distribution of cyclohexane at equilibrium can be estimated (see **Table 3.3**).

Table 3.3 Estimated equilibrium distribution (fugacity model, level I)

Compartment	%
Air	99.98
Water	0.01
Soil	0.002

Based on the physico-chemical properties of cyclohexane, the atmosphere is the preferred target compartment.

Elimination in STPs

Based on the aforementioned physico-chemical properties ($\log H = 4.2$; $\log Pow = 3.44$), as well as the biodegradation rate of 1 h^{-1} in STP, the elimination through biodegradation and distribution can be estimated with the SIMPLETREAT model (**Table 3.4**).

Table 3.4 Estimated distribution in a STP (SIMPLETREAT)

% to air	62.5
% to water	3.4
% to sludge	6.3
% degraded	27.8
% removal	96.6

3.1.1.3 Accumulation

According to the (Q)SAR proposed in EC (1996), a BCF of 167 can be estimated for fish.

A bioaccumulation test with *Cyprinus carpio* according to OECD GL 305 C yielded a BCF of 31-102 at a water concentration of $100 \mu\text{g/l}$ and 37-129 at $10 \mu\text{g/l}$ (CITI, 1992). For the present assessment, a worst-case BCF of 129 will be used.

3.1.2 Aquatic compartment

3.1.2.1 Releases to surface water

3.1.2.1.1 Production

In the TGD (EC, 1996), a generic (i.e. non site-specific) exposure scenario ("Use Category Document", UCD) for the release into surface water of intermediates during production and processing is proposed. This scenario reflects a realistic worst-case situation.

As the production process does not involve water, the releases into wastewater will mainly occur during transformation. Nevertheless, releases into water during production are possible e.g. through pumps or flue gas scrubbing. According to US EPA (1980), plants comprising at least 16% of the total cyclohexane capacity in the USA in 1980 used an aqueous solution to purify the recycled hydrogen from the production process. No release factors are proposed though.

The proposed release factor for the production of a chemical in a dry process is zero (EC, 1993). Releases are nevertheless possible, and specific data on releases at production are available from three producers (see **Table 3.5**):

Table 3.5 Specific data on releases from several producers

ID	Releases	Data used for estimation
A	< 0.1	calculated from known wastewater flows together with average content of total organic carbon; (81 samples in 1992)
G	0.2 t/a	based on average measured concentrations in effluent (60 samples)
H	ca. 1 t/a	estimation based on measurements of COD, BOD and N (Kjeldal) in effluent

Based on the data above, it can be concluded that the overall releases into surface water are low. Given the high releases from other applications, the contribution from production processes is probably negligible, but the data collected from the producers can nevertheless be used in the assessment. The following releases can be assumed for production:

- local: -,
- regional: 1 t/a,
- continental: 1.4 t/a.

For the local exposure assessment, a site-specific evaluation will be performed.

3.1.2.1.2 Use as an intermediate

The TGD (EC, 1996; Table A1.1) proposes a release factor into wastewater of 0.7% during chemical transformation of cyclohexane (i.e. a total of 6,048 t/a, 605 t/a on a regional level and 5,443 t/a on a continental level). Furthermore, a fraction of main source of 0.15 applied to 10% of the total releases is proposed for the local release (EC, 1996; Table B3.2). This would result in the following releases into wastewater:

- local: 90.7 t/a,
- regional: 605 t/a,
- continental: 5443 t/a.

The local release to surface water, assuming an elimination rate in a STP of 96.6%, would be 3.1 t/a.

Specific release data to surface water are available from some European producers, as indicated in **Table 3.6**.

Table 3.6 Releases during use as an intermediate at several sites

ID	Release [t/a]	Specific data used	Fate of sewage sludge
K and AA	< 1.5	daily analytical measurements, concentration in treated effluent never reached the detection limit .	incinerated
	ca. 0.55	release to raw effluent based on water solubility and wastewater stream	incinerated
D	0.023-2.3	effluent flow 130 m ³ /h; concentration in raw effluent ca. 0.02-2 mg/l (extraction and GC 3 samples), no STP on site	no STP
BB	< 0.08	release to surface water, measurement in STP effluent	incinerated
CC	ca. 0.002	release to surface water, measurement in STP effluent	incinerated
DD	< 0.26	release to surface water, measurement in STP effluent (below detection limit)	anaerobic, low production of sludge, reused for inoculating other STPs
EE	1.4-3.6	release to STP, mean concentration in effluent from production unit	used as fertiliser

The total quantity of cyclohexane used on the sites reported in **Table 3.6** is ca. 900,000 t/a, which is close to the total of 864,000 t/a estimated in Section 2 for this use. It can therefore be assumed that specific data on releases are available from all the users of cyclohexane as a chemical intermediate in the EU. An assessment can therefore be performed by using the specific data.

Further data are available from the Toxic Release Inventory from the USA for 1994 (US EPA, 1996). Selecting records declaring “use as a reactant” for “industrial organic chemicals”, surface water releases varied from 0-20 kg/a (10 records). One site declared release into a publicly owned STP of 3.8 t/a.

The total release into surface water, using the figures in **Table 3-6** would be < 4.3 t/a (assuming a default elimination of 96.6% in a STP for those sites where the analytical measurements were performed in the raw sewage). The regional and continental releases would then be:

- regional: 0.43 t/a,
- continental: 3.9 t/a.

The local releases will be assessed for each site. For the estimation of the release into agricultural soil through the use of sewage sludge as fertiliser, a release of 3.6 t/a into sewage sludge will be used as this is the maximum quantity released into a STP from which sewage sludge is used in agriculture.

3.1.2.1.3 Use as a solvent for production processes in chemical industry

According to the TGD (EC, 1996; Table A3.2), the releases into wastewater are estimated to be up to 5% of the amount used (i.e. a total of 900 t/a). It furthermore proposes a fraction of main source of 0.4 applied to 10% of the total emissions for the local release (Table B3.2). The releases into wastewater are therefore estimated at:

- local: 36 t/a,
- regional: 90 t/a,
- continental: 810 t/a.

The local release into surface water, assuming an elimination rate in a STP of 96.6% would be 1.2 t/a.

Information on releases is available from the US Toxic Release Inventory for 1994 and 1995 (US EPA, 1996; 1997). Records declaring use “as a chemical processing aid” and “as a manufacturing aid” in the following industries were selected:

- industrial (in)organic chemicals,
- cyclic organic crudes and intermediates, and organic dyes,
- medicinal products and botanical products,
- pharmaceutical preparations,
- plastics materials, synthetic resins,
- synthetic rubber,
- tyres and inner tubes.

In 1994, among the 46 selected sites, 9 reported surface water releases from 4.5-5,400 kg/a (> 1,000 kg/a at four sites). Three sites declared releases into a publicly owned STP between 112 and 1,740 kg/a. In 1995, among the 66 selected sites, 10 reported surface water releases from 24-2,200 kg/a (> 1,000 kg/a at 2 sites). Seven sites declared releases into a publicly owned STP between 14 and 675 kg/a. Only 5 reported that their estimations were based on monitoring data (0, 0, 0, 76 and 49 kg into surface water), while estimation methods were used for the other sites. No data on the estimation methods or the flow of the receiving river are available.

A survey conducted by the European manufacturers of cyclohexane among users in the USA reporting surface water releases has revealed that:

- cyclohexane is a suitable solvent for some rubber and polymer manufacture,
- it is a suitable solvent for the extraction of fragrances and flavors,
- cyclohexane forms an azeotrope with water and is used in fine chemicals synthesis (esterification, alkylation, etc.),
- cyclohexane is used in proprietary processes and products (in the USA).

Based on the data from the USA, a survey among European users was attempted (APA, 1998). More than 50 companies were contacted. The use of cyclohexane in wet processes was reported from ca. 20 companies and data on releases were made available. In general, rubber manufacturers are the biggest consumers of cyclohexane (up to several 1,000 t/a), while for the production of fine chemicals or for the extraction of fragrances, the consumption is usually less than 100 t/a per site.

The specific data available from European users of cyclohexane as a solvent in chemical reaction processes are presented in **Table 3.7**.

As it would be impossible to identify all users of cyclohexane in the EU within the timeframe usually allocated to the risk assessment of an existing chemical, it has to be assessed whether the available site-specific data are sufficient to overrule the default release estimations. The total quantity of cyclohexane used by the companies which provided specific release data, corresponds to approximately one third of the total quantity estimated for this use. Compared to the number of sites using cyclohexane in the USA, it can be considered that probably also about one third of the number of sites using cyclohexane in the EU are represented in **Table 3.7**. Furthermore, companies producing rubber or other polymers, companies using cyclohexane as an extraction solvent for fragrances, and companies using cyclohexane as an extraction solvent in chemical synthesis are represented in the table below. Although some companies releasing higher amounts of cyclohexane than those listed in **Table 3.7**, a representative worst-case situation can nevertheless be derived from the available information which would overrule the default release scenario.

As can be seen from the table below, the releases into wastewater, when monitored are lower than those estimated above. Only estimations based on water solubility are of the same order of magnitude. The highest monitored release into wastewater is 5 t/a and the highest release into surface water based on monitoring data is 1.85 t/a. This high release into surface water is due to only mechanical treatment on site and no subsequent biological treatment. This may not be representative.

For a generic assessment, a realistic worst-case release into wastewater of 5 t/a will be assumed for this assessment. In summary, the releases into wastewater are estimated at:

- local: 5 t/a,
- regional: 90 t/a,
- continental: 810 t/a.

Table 3.7 Estimations of releases due to industrial use of cyclohexane as solvent in chemical processes at several specific industrial sites

ID	Quantity used	Release to wastewater [t/a]	Release to surface water [t/a]	Estimated PEC [$\mu\text{g/l}$]	Data used	Fate of sewage sludge
A160	ca. 213 t/a	0		0	only atmospheric releases; dry process	incinerated
A144	ca 40 (2 sites)	0			incineration	
		0			incineration	
A166	ca. 50	0			incineration	
A161 ¹⁾	ca. 47 t/a	0		0	only atmospheric releases; dry process	not relevant
A161 ¹⁾	ca. 60 t/a	< 0.016		0.05	monitoring data in the effluent; river at low flow 10 m ³ /s	no STP
A161 ¹⁾	ca. 250 t/a	< 0.06	< 0.002	0.003	monitoring in treated effluent, release to a second municipal STP, default removal in second STP, default dilution factor	?
A101		0.08		3.4	monitoring in the effluent; default STP removal and dilution	incinerated
A103		5	< 0.25	< 3.8	monitoring in the untreated effluent; stripping efficiency of STP for cyclohexane measured to be > 98%; river at low flow 2.1 m ³ /s	Land filled
A118			< 1.7	< 4.5	monitoring of VOC in effluent (assumed to be 100% cyclohexane), release to sea, dilution based on measurement of oils at 50 m from point of discharge is > 220	no sludge, mechanical treatment only
A136		< 0.2	< 0.007	< 0.1	monitoring data in untreated effluent, default STP removal, dilution in STP and receiving river	used as fertiliser in agriculture
A137	ca. 90	< 0.39	< 0.013	< 0.0004	monitoring of hydrocarbons in untreated effluent (more than 90% aromatics), default STP removal, river at low flow 1,100 m ³ /s	Land filled
A148			< 0.003	< 4	monitoring of hydrocarbon content (assumed to be 100% cyclohexane), low dilution in river	controlled discharge

Table 3.7 continued overleaf

Table 3.7 continued Estimations of releases due to industrial use of cyclohexane as solvent in chemical processes at several specific industrial sites

ID	Quantity used	Release to wastewater [t/a]	Release to surface water [t/a]	Estimated PEC [$\mu\text{g/l}$]	Data used	Fate of sewage sludge
A163	ca. 3,500		1.85	3.5	monitoring in the treated effluent, only mechanical treatment, river at low flow 17 m ³ /s	no sludge, mechanical treatment only
A165	ca. 30	< 0.182	< 0.006	0.85	monitoring of hydrocarbon content in effluent (assumed to be 100% cyclohexane), release into municipal STP, default elimination rate and flow of STP, default dilution factor	?
A110	ca. 1,000	ca. 1.1	ca. 0.04	0.001	measured concentration of hydrocarbons in untreated effluent. Cyclohexane roughly 25% of hydrocarbons. Default STP removal, river at low flow 1,100 m ³ /s	incinerated
A104		50-80	1.7-2.7	< 0.8	worst-case estimate based on water solubility and wastewater flow; default STP removal; river at low flow 110 m ³ /s	?
A160	ca. 315 t/a	< 1.5			estimated via water solubility of cyclohexane and volume of generated wastewater	incinerated
A112	ca. 45	max 21	max. 0.72	max. 0.2	worst-case estimate based on water solubility and wastewater flow; default STP removal; river at low flow 110 m ³ /s	incinerated

1) Same company, different sites

3.1.2.1.4 Formulation and use of adhesives and coatings

Formulation

According to the TGD (EC, 1996; Table A2.1), the releases into wastewater are estimated to be 0.3% of the amount used (i.e. a total of 54 t/a). It furthermore proposes a fraction of main source of 0.8 applied to 10% of the total emissions for the local release (Table B2.10). The releases into wastewater are therefore estimated at:

- local: 4.3 t/a,
- regional: 5.4 t/a,
- continental: 48.6 t/a.

The local release to surface water, assuming an elimination rate in a STP of 96.6% would be 0.15 t/a.

Use of adhesives and coatings

According to the TGD (EC, 1996; Table A3.15), the releases to wastewater are estimated to be 2% of the amount used (i.e. a total of 360 t/a). It furthermore proposes a fraction of main source of 0.1 applied to 10% of the total emissions for the local release (Table B 3.13). The releases into wastewater are therefore estimated at:

- local: 3.6 t/a,
- regional: 36 t/a,
- continental: 324 t/a.

The local release to surface water, assuming an elimination rate in a STP of 96.6% would be 0.12 t/a.

Further data are available from the US Toxic Release Inventory from the USA (US EPA, 1996). Records declaring use “as a formulation aid” and “as a processing aid” in the following industries were selected:

- adhesives and sealants,
- chemicals and chemical preparations,
- coated and laminated paper,
- metal cans,
- packaging paper and plastics film, coated and laminated,
- paints, varnishes and rubber,
- plastic film and sheet.

Among the 55 selected sites, only one reported surface water releases of 112 kg/a. One further site declared release into a publicly owned STP of 540 kg/a. All the other sites reported that no release occurred with wastewater.

These data would suggest that the estimations proposed in the TGD (EC, 1996) correspond to worst cases and that in over 90% of the sites, the releases to wastewater are negligible.

3.1.2.1.5 Emissions not covered by the life-cycle of the produced/imported substance

Releases are possible from petroleum products naturally containing cyclohexane. Emissions would mainly occur from the marketing and distribution of gasoline and from gasoline powered vehicles. Releases are diffuse and occur mainly to air. Releases into wastewater and surface water are probably negligible. No quantitative data are available to derive PEC values.

3.1.2.2 Estimation of local aquatic concentrations

3.1.2.2.1 Production

Data are available from most producers to allow site-specific evaluations. The results are summarised in **Table 3.8**.

Table 3.8 Estimation of local aquatic concentrations at five specific production sites

ID	PEC _{local} [µg/l]	Specific data used
A	0.06	release of less than 100 kg/a with wastewater stream. Emissions are calculated from known wastewater flows together with analysis of random test samples. A default flow of the receiving river of 60 m ³ /s is assumed as no specific data is available.
B	< 0.03	in the 7 samples (24h mixing samples) taken from the raw effluents of the production facility, the concentration of cyclohexane was below the detection limit of 0.01 mg/l. The default elimination rate of 96.6% in a STP and, a default dilution factor of 10 is used.
F	< 1.00	in the 186 samples (24h mixing samples) taken from the effluents of the production facility, the concentration of cyclohexane was below the detection limit of 0.01 mg/l. As information the flows of the wastewater and the receiving river is missing, a default dilution factor of 10 is used.
G	0.18	release of less than 300 kg/a with wastewater stream. A default flow of the receiving river of 60 m ³ /s is assumed as no specific data is available. The estimation method is not clear and will have to be checked.
H	0.08	release of 1 t/a with wastewater with a wastewater stream of 1,000 m ³ /h. Release estimation based on COD; BOD; N(Kjeldal) measurements in the effluents. The average flow of the receiving river is 1,500 m ³ /s. The 10-percentile flow of the river is not known and is estimated to be 1/3 of the average i.e. 500 m ³ /s.

The above local concentrations are calculated by simple dilution in the receiving river flow. The elimination by adsorption onto suspended matter can be considered to be negligible. For the risk characterisation, a PEC_{local} of < 1 µg/l will be retained.

3.1.2.2.2 Use as an intermediate

The local releases are based on the site-specific data available for all the installations using cyclohexane as an intermediate. The results are summarised in **Table 3.9**.

Table 3.9 Estimation of local aquatic concentrations at several specific transformation sites

ID	PEC _{local} [µg/l]	Specific data used
K and AA	< 0.06	total release of < 1.5 t/a with wastewater (estimation based on daily analytical measurement); low river flow: ca. 1,000 m ³ /s
	0.35	total release of ca. 0.55 t/a with wastewater (estimation based on water solubility and wastewater stream); default river flow of 60 m ³ /s
D	< 0.5	direct release to sea; no STP available; concentration in effluent ca. 0.02-2 mg/l (extraction and GC; 5 samples), release is 800 meters from the shore and at 8 meters of depth Concentration measured at 25, 50 and 100 m from the outlet and were below detection limit of 0.5 µg/l
BB	0.0024	monitoring in STP effluent; dilution factor at low flow ca. 4,120
CC	0.00047	monitoring in STP effluent, dilution at low flow ca. 700
DD	< 0.02	monitoring in STP effluent, dilution at low flow ca. 50,000
EE	0.22-0.56	monitoring in raw effluent, default elimination rate of 96.6%, flow of receiving river at low flow ca. 25,000 m ³ /hr

The highest estimated concentration of 0.56µg/l will be used in the risk characterisation.

3.1.2.2.3 Use as a solvent for production processes in the chemical industry

Using the local release estimated under Section 3.1.2.1.3 of 5 t/a, and using the following default parameters according to the TGD (EC, 1996) (see also EUSES output ⁴),

No of days of operation:	365 d (based on monitoring data during release)
Flow of STP	2,000 m ³ /d
Removal in STP:	96.6% (see Table 3.4)
Dilution	10

a PEC_{microorganisms} of 232 µg/l and a PEC_{local} of 23.2 µg/l can be estimated (cf. Annex 1)

Specific results are available from several sites, as shown in **Table 3.10**.

Table 3.10 Estimations of releases due to industrial use of cyclohexane as a solvent in chemical processes at several specific industrial sites

ID	Estimated PEC [µg/l]	Data used
A160	0	only atmospheric releases; dry process
A144	0	incineration
	0	incineration
A166	0	incineration
A161J	0	only atmospheric releases; dry process
A161	0.05	monitoring data in the effluent; river at low flow 10 m ³ /s

Table 3.10 continued overleaf

⁴ See Euses Calculations on the website of the European Chemicals Bureau: <http://ecb.jrc.it>

Table 3.10 continued Estimations of releases due to industrial use of cyclohexane as a solvent in chemical processes at several specific industrial sites

ID	Estimated PEC [$\mu\text{g/l}$]	Data used
A161	0.003	monitoring in treated effluent, release to a second municipal STP, default removal in second STP, default dilution factor
A101	3.4	monitoring in the effluent; default STP removal and dilution
A103	< 3.8	monitoring in the untreated effluent; stripping efficiency of STP for cyclohexane measured to be > 98%; river at low flow 2.1 m ³ /s
A118	< 4.5	monitoring of VOC in effluent (assumed to be 100% cyclohexane), release to sea, dilution based on measurement of oils at 50 m from point of discharge is > 220
A136	< 0.1	monitoring data in untreated effluent, default STP removal, dilution in STP and receiving river
A137	< 0.0004	monitoring of hydrocarbons in untreated effluent (more than 90% aromatics), default STP removal, river at low flow 1,100 m ³ /s
A148	< 4	monitoring of hydrocarbon content (assumed to be 100% cyclohexane), low dilution in river
A163	3.5	monitoring in the treated effluent, only mechanical treatment, river at low flow 17 m ³ /s
A165	0.85	monitoring of hydrocarbon content in effluent (assumed to be 100% cyclohexane), release into municipal STP, default elimination rate and flow of STP, default dilution factor
A110	0.001	measured concentration of hydrocarbons in untreated effluent. Cyclohexane roughly 25% of hydrocarbons. Default STP removal, river at low flow 1,100 m ³ /s
A104	< 0.8	worst-case estimate based on water solubility and wastewater flow; default STP removal; river at low flow 110 m ³ /s
A112	max. 0.2	worst-case estimate based on water solubility and wastewater flow; default STP removal; river at low flow 110 m ³ /s

As stated above, the site-specific data can be considered representative of the use of cyclohexane as a solvent in chemical industry. The highest estimated surface water concentration based on site-specific data of 4.5 $\mu\text{g/l}$ can therefore be used for the risk characterisation.

3.1.2.2.4 Formulation and use of adhesives and coatings

As shown in Section 3.1.2.1.4, the releases to wastewater during the formulation and use of cyclohexane containing products are negligible, and no local concentrations need to be estimated.

3.1.2.3 Regional concentration in surface water

The regional concentration, as calculated with a level III fugacity model, and taking into account all releases, is (cf. Annex 1):

$$\text{PEC}_{\text{regional aqua}} = 0.05 \mu\text{g/l.}$$

3.1.2.4 Monitoring data

Data on analytical measurements in effluents or surface waters are very scarce. The results are summarised in **Table 3.11**.

Table 3.11 Monitoring of cyclohexane in effluents or surface water

Location	Year	Results ($\mu\text{g/l}$)	Remarks	Reference
The Netherlands, river Rhine	1978	0.03-1	2 locations n = ?	Morra et al. (1979)
Japan, surface water	1979	< 0.2	n = 27 no positive results d.l.: 0.05-0.2 $\mu\text{g/l}$	Environment Agency (1996)
USA, 14 heavily industrialised river basins	1977	0-4	n = 204 13 positive results d.l.: 1 $\mu\text{g/l}$	US EPA (1977)
USA, Gulf of Mexico	1977	open sea: 0-0.003 coastal sea: 0.003-0.02	n = 6 n = 4	Sauer (1981a)
USA, Embarras river, crude oil production area	1975-1977	average: 0.6 maximum: 2.3	n = 16 d.l.: 0.1 $\mu\text{g/l}$	Siefker and Catt (1980)
UK, Mersey estuary and its freshwater inputs	1987/88	freshwater: positive: 3/17 suspended matter: 3/17 estuary: positive: 9/23 suspended matter: 16/23	qualitative results only, no detection limit given	FWR (1990)
USA, plume of a hydrocarbon venting system from an oil platform	1981	0.4	n = 1	Sauer (1981b)
USA, wastewater from an oil platform	1981	100	n = 1	Sauer (1981b)
USA, effluents from chemical manufacturing sites	1976-1978	3 positive results at concentrations < 10 $\mu\text{g/l}$	n = 63	Perry et al. (1979)

Some results from the monitoring data are coherent with the estimated concentrations, but they are generally lower. They are too scarce to be representative and cannot be used to overrule the estimated PECs.

3.1.2.5 Sediment

Cyclohexane was not detected in 27 sediment samples from surface waters in Japan in 1979. The detection limit was 0.1-0.4 $\mu\text{g/kg dw}$.

As no positive results from monitoring programmes on cyclohexane concentrations in sediment or experimental results with benthic organisms are available, a risk assessment cannot be performed for this compartment. As the chemical is only moderately hydrophobic, it can be assumed that the risk assessment for sediment is covered by the risk assessment for surface water.

3.1.3 Atmosphere

3.1.3.1 Releases to atmosphere

3.1.3.1.1 Production

The atmospheric release during production proposed by the TGD (EC, 1996) is 1%, i.e. 10 kg per tonne produced.

The US EPA (1980) reports emission factors for the production of cyclohexane as follows (the figures refer to kg of Volatile Organic Compounds (VOC) released per tonne cyclohexane produced):

Uncontrolled emissions from a 150,000 t/a capacity plant:

fugitive emissions:	0.47 kg VOC/t cyclohexane
storage emissions:	1.31 kg VOC/t cyclohexane
handling emissions:	0.15 kg VOC/t cyclohexane

Controlled emissions from a 150,000 t/a capacity plant:

fugitive emissions:	0.11 kg VOC/t cyclohexane
storage emissions:	0.20 kg VOC/t cyclohexane
handling emissions:	0.15 kg VOC/t cyclohexane

The US EPA (1980) reported that the fugitive emissions would include benzene, cyclohexane, methane and other hydrocarbons. The benzene content of the fugitive emissions was reported to be about 10% of the total VOC emission. A similar content for cyclohexane can be assumed here.

The US EPA (1980) reported that storage emissions would be mainly from benzene and cyclohexane storage. The benzene content of the storage emissions was reported to be about a third of the total VOC emission. A similar content for cyclohexane can be assumed here.

Handling emissions were estimated by the US EPA (1980) to arise almost exclusively from the transfer of cyclohexane to transport vessels for removal from the production plant. 90% cyclohexane content for this emission can be assumed.

Using these assumptions of cyclohexane content, the emission factors become:

Uncontrolled emissions from a 150,000 t/a capacity plant:

fugitive:	0.05 kg cyclohexane/t produced
storage:	0.44 kg cyclohexane/t produced
handling:	0.14 kg cyclohexane/t produced
total:	0.63 kg cyclohexane/t produced

Controlled emissions from a 150,000 t/a capacity plant:

fugitive:	0.01 kg cyclohexane/t produced
storage:	0.07 kg cyclohexane/t produced
handling:	0.14 kg cyclohexane/t produced
total:	0.22 kg cyclohexane/t produced

This would correspond to uncontrolled emissions of 94.5 t/a and controlled emissions of 33 t/a at a 150,000 t/a capacity plant.

Several European producers have provided release estimations, and these are presented in **Table 3.12**.

Table 3.12 Estimation of local releases at production sites in the EU

ID	Quantity produced [t/a]	Release [t/a]	Emission factor kg/tonne produced	Precision on estimation
A	100,000-200,000	1.92	0.01-0.02	estimation of fugitive emissions only with a national method;
B	50,000-100,000	< 62	< 0.62-1.24	estimation for fugitive emissions with VDI methods 3,479 and 2,440; figures for 1994; in 1990 and 1992 they were, respectively <115 t and < 95 t.
F	200,000-300,000	230	0.76-1.15	estimated release due to tank respiration with American Petroleum Institutes methods API2518 and API2517; closed system, there are no release of flue gases from the production process
G	< 100,000	ca. 100	ca 1.0	release from production and storage; the determination method is not clear yet and has to be checked
H	100,000-200,000	10	0.05-0.10	estimated release due to tank respiration; estimation for fugitive emissions with a national method; closed system, there are no release of flue gases from the production process
J	< 100,000	18.5	> 0.185	release due to tank respiration; the determination method is not clear yet and has to be checked; flue gases from production processes are incinerated.

The above data suggest that the emission factor for uncontrolled emissions of 0.63 kg/tonne produced proposed by the US EPA (1980) realistically describes the emissions at European production sites. For local and regional releases, the highest emission reported in **Table 3.12** can be used. In summary the estimated releases into the atmosphere during production are:

- local: 230 t/a,
- regional: 230 t/a,
- continental: 544 t/a.

3.1.3.1.2 Use as an intermediate

The atmospheric release during production proposed by the ETGD (EC, 1996; Table A3.3, continuous production) is 0.1%, i.e. 1 kg per tonne used. No further data on release factors were found in the literature.

Several European users have provided release estimations, and these are presented in **Table 3.13**.

Table 3.13 Estimation of local releases at sites using cyclohexane as an intermediate in the EU

ID	Quantity produced [t/a]	Release [t/a]	Emission factor kg/tonne produced	Specific data used
D	< 100,000	500	> 5	actual release of ca. 500 t/a from the oxidation reactor stack vent; the determination method is not clear yet and has to be checked
K	ca. 200,000–300,000	ca. 60	0.2-0.3	actual release of ca. 60 t/a (based on analytical measurements)
AA	ca. 200,000-300,000	34.7	0.11-0.17	actual release of 34.7 t/a including the release from on-site solvent use (based on analytical measurements)

Further data are available from the US Toxic Release Inventory (US EPA, 1996). Selecting records declaring “use as a reactant” for “industrial organic chemicals”, surface water releases varied from 0.225-140 t/a (10 records). Only 4 sites reported releases of more than 50 t/a.

For an overall release, the release factor of 1 kg/tonne used as proposed in the EU (1996) seems to be realistic. For local and regional releases, the highest release of 500 t/a from **Table 3.13** will however be used:

- local: 500 t/a,
- regional: 500 t/a,
- continental: 864 t/a.

3.1.3.1.3 Use as a solvent for production processes in the chemical industry

According to the TGD (EC, 1996; Table A3.2), the releases into air are estimated to be up to 95% of the amount used (i.e. a total of 17,100 t/a). Furthermore, it proposes a fraction of main source of 0.4 applied to 10% of the total emissions for local release (Table B3.2). The releases into air are therefore estimated at (see Annex 1):

- local: 684 t/a,
- regional: 1,710 t/a,
- continental: 15,400 t/a.

Some specific data are available from several European users of cyclohexane as a solvent in chemical reaction processes (see **Table 3.14**).

Table 3.14 Estimations of releases due to the industrial use of cyclohexane as solvent in chemical processes at several specific industrial sites

ID	Quantity used	Release [t/a]	Data used
A160	ca. 213 t/a	19	based on analytical measurements; data from 1992-1993
A160	ca. 315 t/a	4	based on analytical measurements; data from 1992-1993
A161	ca. 60 t/a	ca.44	the estimation method is not clear yet and has to be checked;
A161	ca. 47 t/a	35.6	the remaining amount is incorporated in the finished products
A161	ca. 250 t/a	237.5	the 250 t/a is the fraction completely lost during the process; the compartment of release is not clear; a default of 95% to air would represent 237.5 t/a;

Further data are available from the US Toxic Release Inventory (US EPA, 1996). Records declaring use “as a chemical processing aid” and “as a manufacturing aid” in the following industries were selected:

- industrial (in)organic chemicals,
- cyclic organic crudes and intermediates, and organic dyes,
- medicinal products and botanical products,
- pharmaceutical preparations,
- plastics materials, synthetic resins,
- synthetic rubber,
- tyres and inner tubes.

Among the 46 selected sites, all reported air releases from 0.005-400 t/a. Four sites reported releases of more than 100 t/a.

These data tend to confirm the initial estimation proposed in the TGD (EC, 1996). The releases into air used in this assessment are therefore:

- local: 684 t/a,
- regional: 1,710 t/a,
- continental: 15,400 t/a.

3.1.3.1.4 Formulation and use of adhesives and coatings

Formulation

According to the TGD (EC, 1996; Table A2.1), the releases into air are estimated to be 2.5% of the amount used (i.e. a total of 450 t/a). Furthermore, it proposes a fraction of main source of 0.8 applied to 10% of the total emissions for local release (Table B2.10). The releases into air are therefore estimated at (see Annex 1):

- local: 36 t/a,
- regional: 45 t/a,
- continental: 405 t/a.

Use of adhesives and coatings

According to the TGD (EC, 1996; Table A3.15), the releases into air are estimated to be 90% of the amount used (i.e. a total of 16,200 t/a). Furthermore, it proposes a fraction of main source of 0.1 applied to 10% of the total emissions for the local release (Table B3.13). The releases into air are therefore estimated at (see Annex 1):

- local: 162 t/a,
- regional: 1,620 t/a,
- continental: 14,580 t/a.

Further data are available from the US Toxic Release Inventory (US EPA, 1996). Records declaring use “as a formulation aid” and “as a processing aid” in the following industries were selected:

- adhesives and sealants,
- chemicals and chemical preparations,

- coated and laminated paper,
- metal cans,
- packaging paper and plastics film, coated and laminated,
- paints, varnishes and rubber,
- plastic film and sheet.

Among the 55 selected sites, almost all reported air releases between 0.005 and 127 t/a. One site reported releases of more than 100 t/a, while 5 sites reported releases of more than 50 t/a.

These data confirm the estimations proposed in the TGD (EC, 1996) and the above figures can be used for the risk assessment.

3.1.3.1.5 Emissions not covered by the life-cycle of the produced/imported substance

A review of available emission data and an estimation of releases in the UK were performed by Nielsen and Howe (1995). The results are summarised below.

Emissions from gasoline marketing and dispersions

This category of emissions covers the releases of cyclohexane and other Volatile Organic Compounds (VOC) occurring when gasoline is transferred from the refinery (place of production) to service stations and from service stations to individual vehicles. It is assumed that gasoline composition contains about 0.3% cyclohexane.

The estimated releases for the UK range between 228 and 401 tonnes per year. Assuming the same per capita gasoline consumption within all of the EU, an extrapolated release in the EU of 1,442–2,536 t/a can be estimated.

Emission from gasoline-powered vehicles

Cyclohexane is a component of the gasoline used in motor vehicles and is released into the atmosphere in the exhaust gases from these vehicles. Cyclohexane is also released into the environment due to evaporative losses from vehicles. These losses can occur in three ways:

- when the vehicle is being driven (running losses),
- when the vehicle is stationary after being driven, the engine is hot (hot soak losses), and
- when the vehicle is stationary and subject to day/night temperature changes (diurnal losses).

The estimated releases for the UK range between 2,565 and 4,010 tonnes per year. Assuming the same per capita gasoline consumption within all of the EU, an extrapolated release in the EU of 16,160–25,360 t/a can be estimated.

Under worst-case assumptions, the total releases from the handling and use of fuel can be estimated as:

- regional: 2,790 t/a,
- continental: 25,100 t/a.

3.1.3.2 Estimation of local air concentrations and deposition rates

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

$$PECl_{\text{local air}} (\text{mg/m}^3) = E_{\text{local air}} \cdot C_{\text{std air}}$$

where $E_{\text{local air}}$ (kg/d) = local direct emission rate into air
 $C_{\text{std air}}$ = standard concentration in air at source strength of 1 kg/d
 $= 2.78 \cdot 10^{-4} \text{ mg/m}^3$.

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

$$DEP_{\text{total}} = (E_{\text{local air}} + Estp_{\text{air}}) \cdot DEP_{\text{std gas}}$$

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

Table 3.15 presents the local air concentrations as well as the average deposition rates. The deposition rates take account of the indirect releases through stripping from the STP (see also Annex 1).

Table 3.15 Local concentrations in air during emissions, average air concentrations and average deposition rates

Life- stage	PECl _{local air} [µg/m ³]	Average air concentration [µg/m ³]	DEP _{total} [mg/m ² /d]
Production	213	175	0.189
Use as an intermediate	463	381	0.415
Use as a solvent in chemical industry	1,060	521	0.581
Formulation of adhesives and coatings	33	27	0.030
Use of adhesives and coatings	146	123	0.130

Air measurements are available from one European production site (B). The average concentration in 1994 at 14 sampling locations on the production site (195 samples) was 2-10 µg/m³ (personal communication).

3.1.3.3 Local concentration due to car exhausts

Local concentrations due to car exhausts can be estimated with computer models, e.g. the CAR-model (Eerens et al., 1993).

The average exhaust hydrocarbon composition was determined by Nelson and Quigley (1984). A representative set of 67 vehicles was chosen and their exhaust was sampled with a dynamometer gas sampling system according to a US Federal Test Procedure including a cold transient phase, a cold stabilised phase and a hot stabilised phase of the engine. The average cyclohexane content compared to the total hydrocarbon content was determined to be 0.6% (w/w). In a further study (Nelson et al., 1983), the cyclohexane content in petrol was analysed in 115 separate petrol samples and the cyclohexane content in the petrol vapour was estimated according to Raoult's

law. The average cyclohexane content compared to the total hydrocarbon content was determined to be 0.3% (w/w). As an initial approximation, a cyclohexane content of 0.6% (w/w) in total VOC emissions from cars can be assumed.

The following assumptions have been used:

- annual average wind velocity outside the urban environment at a height of 10m: 4 m/s,
- a road with 10,000 vehicles per day of which 5% heavy vehicles,
- background concentration is $0 \mu\text{g}/\text{m}^3$,
- emission factor of cyclohexane is 0.006 times the emission factor of VOC (cf. above),
- emission factors for VOC are for the average car in the Netherlands in 1996 (in g/km).

		Light vehicles	Heavy vehicles
Urban traffic	exhaust	1,554	6,250
	evaporation	1,265	0,007
Motorway	exhaust	0,471	1,739
	evaporation	0,050	0,000

The following annual average concentrations in $\mu\text{g}/\text{m}^3$ can be derived for cyclohexane:

Type of road			Distance [m]					
			5	10	15	20	25	30
Urban	1	exhaust	0,213	0,167	0,127	0,093	0,065	0,043
		evaporation	0,143	0,112	0,085	0,063	0,044	0,029
	2	exhaust	0,383	0,278	0,197	0,140	0,107	0,098
		evaporation	0,257	0,187	0,132	0,094	0,072	0,066
	3A	exhaust	0,459	0,338	0,242	0,171	0,125	0,105
		evaporation	0,308	0,227	0,162	0,115	0,084	0,070
	3B	exhaust	0,689	0,507	0,362	0,256	0,188	0,157
		evaporation	0,463	0,340	0,244	0,172	0,126	0,106
	4	exhaust	0,659	0,472	0,324	0,214	0,144	0,112
		evaporation	0,443	0,317	0,218	0,144	0,097	0,075
Motorway	1	exhaust	0,063	0,050	0,038	0,028	0,019	0,013
		evaporation	0,006	0,004	0,003	0,002	0,002	0,001
	2	exhaust	0,114	0,083	0,059	0,042	0,032	0,029
		evaporation	0,010	0,007	0,005	0,004	0,003	0,003

Road types:

- 1 road through open field, no or very few buildings,
- 2 base type, all roads different from other types,
- 3A roads with buildings on both sides, distance from road axis

- 3B less than 3 times the height of the buildings,
roads with buildings on both sides, distance from road axis
less than 1.5 times the height of the buildings,
- 4 roads with buildings on one side, distance from road axis
less than 3 times the height of the buildings.

In general these concentrations are lower than those estimated in the vicinity of plants producing, processing or employing cyclohexane.

3.1.3.4 Regional concentration in air

The regional concentration, as calculated with a level III fugacity model, taking into account all releases is (cf. Annex 1):

$$PEC_{\text{regional air}} = 0.35 \mu\text{g}/\text{m}^3$$

3.1.3.5 Monitoring in the atmosphere

The available data on occurrence of cyclohexane are summarised in **Table 3.16**.

Table 3.16 Monitoring of cyclohexane in the atmosphere

Location	Year	Results ($\mu\text{g}/\text{m}^3$)	Remarks	Reference
Oxfordshire, UK 30 m from an infrequently used road	5/1986-3/1987	< 0.14-1.43	n = 127 77 samples below d.l.	Jones (1988)
The Netherlands, in the vicinity of over 300 homes in Ede and Rotterdam	1981-1983	median: 0.4 max.: 2.0	n > 300	Lebret et al. (1986)
Austria, region of Vienna	10/1986-2/1987	urban (street level): 6.21 urban (elev. 52 m): 2.72 suburbs: 1.75 semi-rural: 0.68	n = 17 (average) n = 12 (average) n = 16 (average) n = 9 (average)	Lanzerstorfer and Puxbaum (1990)
Norwegian Arctic	July 1982 March 1983	< 0.07 (d.l.) 0.189	n = 9 n = 10 (average)	Hov et al. (1984)
USA, Houston, Texas	1973/1974	downtown Houston: 10-19 industrial area: 0-130.3	n = 2 n = 14	US EPA (1979a)
USA, Texas, State Forest	1978	range: 0.3-3.2 average: 1.58	n = 14	US EPA (1979b)
USA, Los Angeles	Sept./Nov. 1981	range: 24.4-108.2	n = 23	Grosjean and Fung (1984)
USA, urban, industrial & rural areas	1980	urban: 0-3.2 oil shale devel. area: 0.1-0.6 rural area: 0-1.2 refinery: 8.7; 14.4 nat. gas facility: 31	n = 6 n = 5 n = 9	Arnts and Meeks (1981)
USA, 7 cities		range: 0.35-108.2		Beals et al. (1986)

Table 3.16 continued overleaf

Table 3.16 continued Monitoring of cyclohexane in the atmosphere

Location	Year	Results ($\mu\text{g}/\text{m}^3$)	Remarks	Reference
USA, 39 cities	1984-1986	range: 0.19-396.7 median: 2.13 75-percentile: 4.7	n > 800 sampling June - Sept. each year	Seila and Lonneman (1988)
USA, New Jersey, inside road tunnel	1972 1982	average: 55.6 average: 44.2	n = 2 n = 28	Lonneman et al. (1986)
Australia, Sydney downwind of refinery complex and downwind of business district	1979/1980	average: 3.14	3 sites, n = 140	Nelson and Quigley (1982)
Japan, Tokyo, flight path to airport, altitude 350-600 m	1980	average: 0.35	n = 66	Uno et al. (1985)

The estimated regional concentration corresponds well with measured concentrations in rural areas. In areas with high road traffic, the measured concentrations are much higher (about $1\text{-}10\ \mu\text{g}/\text{m}^3$). They are even higher than those estimated by modelisation (about $0.1\text{-}1\ \mu\text{g}/\text{m}^3$). The concentrations measured in the vicinity of a refinery or a natural gas facility approach the local concentrations estimated for the industrial use of cyclohexane.

3.1.4 Terrestrial compartment

3.1.4.1 Estimation of local soil and groundwater concentrations

The release of cyclohexane to soil is expected to occur through atmospheric deposition after local release to the atmosphere at the production, formulation and processing sites. Furthermore the input through sludge application on agricultural soil will be taken into account.

The estimation is performed as proposed in the TGD (1996) with the release to STPs and the atmospheric deposition rates derived above (for details see Annex 1). The results are summarised in **Table 3.17**.

Table 3.17 Local concentrations in soil and groundwater

Life- stage	PEC _{local soil} [$\mu\text{g}/\text{kg wwt}$]	C _{grw} [$\mu\text{g}/\text{l}$]
Production	0.9	0.06
Use as an intermediate	63	0.83
Use as a solvent in chemical industry	86	1.15
Formulation of adhesives and coatings	0.14	0.009
Use of adhesives and coatings	0.6	0.04

3.1.4.2 Regional concentration in soil and groundwater

The regional concentration, as calculated with a level III fugacity model, taking into account all releases is (cf. Annex 1):

$$PEC_{\text{regional}}^{\text{soil}} = 0.005 \text{ } \mu\text{g/kg wwt}$$

$$PEC_{\text{regional}}^{\text{grw}} = 0.0004 \text{ } \mu\text{g/l}$$

3.1.4.3 Monitoring data

No results of monitoring data in soil are available.

Groundwater

A groundwater investigation was performed in 1992 at the immediate vicinity of a transformation site using high quantities of cyclohexane as an intermediate and releasing ca. 500 t/a into the atmosphere. Samples were taken at two depths (15 and 25 m) from 8 drilling wells. No cyclohexane was found in any of the samples (the detection limit is not clear and is thought to be 1 $\mu\text{g/l}$) (personal communication).

A survey was carried out in the UK of 32 public and private supply boreholes in three major British aquifer systems in areas of sandstone, limestone and chalk. Cyclohexane was one of the most commonly occurring compounds found in the 32 samples collected. The overall average concentration was 0.022 $\mu\text{g/l}$ and the maximum concentration was 0.08 $\mu\text{g/l}$ (Kenrick et al., 1995).

These concentrations lie within the range of estimated local concentrations.

3.1.5 Secondary poisoning

As cyclohexane is not classified as “Toxic” or “Harmful with at least R48 or R60-R64, a risk characterisation for secondary poisoning is not required.

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

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Toxicity test results

In the following, the most relevant results from acute toxicity tests with aquatic organisms are presented.

Vertebrates

Pimephales promelas 96h LC50 4.53 mg/l
(flow-through, measured concentrations), (Geiger et al., 1987)

Many other results are available, but mostly performed in static open systems without analytical monitoring (Mattson et al., 1976; Pickering and Henderson, 1966; Koenemann, 1981; Juhnke and Lüdemann, 1978; Benville et al., 1985; CITI, 1992). Due to the high volatility of cyclohexane, they therefore cannot be considered as valid and are not reported here.

Invertebrates

Fresh-water species

Daphnia magna 48h EC50 3.78 mg/l
(static, closed system without headspace, nominal concentration), (Abernethy et al., 1986)

Daphnia magna 48h EC50 0.9 mg/l
(static, closed system, low headspace, based on initial measured concentrations, average of 3 test series), (Adema and van den Bos Bakker, 1986)

The test performed by Abernethy et al. (1986) is dependant on water solubility as test solutions were prepared by successive dilution of saturated solutions and the concentrations derived from known solubility in water. As initial test concentrations were measured in the test by Adema and van den Bos Bakker (1986), these results are preferentially used in the risk assessment.

Further tests have been performed by Das and Konar (1988) in open static systems and are therefore not considered in this risk assessment.

Marine species

Crangon franciscorum 96h EC50 1.9 mg/l
(static; measured concentration, not clear whether open or closed system), (Benville et al., 1985)

Artemia sp. 24h EC50 7.32 mg/l
(static; closed system without headspace, nominal concentration), (Abernethy et al., 1986)

Cheatogammarus marinus 96h LC50 2.2 mg/l
(semi-static; closed system, low headspace, based on initial measured concentrations), (Adema and van den Bos Bakker, 1986)

Mysidopsis bahia 96h LC50 2.2 mg/l
(semi-static; closed system, low headspace, based on initial measured concentrations), (Adema and van den Bos Bakker, 1986)

Plants

Several experimental test results with algae are available (Hutchinson et al., 1980; BASF AG, 1990; Bringmann and Kühn, 1977), with EC50 values between 31.9 and > 500 mg/l. These test results were not considered to be valid as the volatile nature of cyclohexane was not sufficiently taken into account in these tests. An additional test, under closed conditions was performed:

<i>Selenastrum capricornutum</i>	72h EbC50	3.4 mg/l
	72h NOEbC	0.9 mg/l
	72h E μ C50	> 4.4 mg/l
	72h NOE μ C	0.94 mg/l

(measured concentration), (Exxon 1998)

Bacteria and protozoa

Tetrahymena elliotti 24h TT 24.2 mg/l
(TT: intermediate concentration between lowest and highest concentration causing cell death and permitting cell replication, respectively; nominal concentration, closed test system, no headspace) (Rogerson et al., 1983)

Methanogenic bacteria 24h EC50 150 mg/l
(effect: inhibition of gas formation; nominal concentrations corrected for headspace of closed test tubes) (Blum and Speece, 1991)

Aerobic heterotrophic bacteria of activated sludge 15h EC50 29 mg/l
(effect: inhibition oxygen consumption; nominal concentrations corrected for headspace of closed test tubes) (Blum and Speece, 1991)

Nitrosomas sp. 24h EC50 97 mg/l
(effect: inhibition of ammonia use; nominal concentrations corrected for headspace of closed test tubes) (Blum and Speece, 1991)

Other test results are available, which are either not relevant for risk assessment or where inadequate test systems were used (Bringmann and Kühn, 1977; Bringmann and Kühn, 1980; Zhao et al., 1993).

3.2.1.2 Comparison with (Q)SAR data

Considering the chemical structure of cyclohexane, it can be assumed that it acts by non-polar narcosis. Using the (Q)SAR-relationships proposed in the TGD for base-line toxicity, the following effect concentrations can be estimated:

Fish

<i>Pimephales promelas</i>	96h LC50	4.1 mg/l
	28-32d NOEC	0.3 mg/l

Invertebrates

<i>Daphnia magna</i>	48h EC50	2.2 mg/l
	16d NOEC	0.3 mg/l

Algae

<i>Selenastrum capricornutum</i>	72-96h EC50	1.8 mg/l
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These estimations are very consistent with the actual determined concentrations and only differ by a factor of approx. 2. The agreement between the predicted and measured values confirms that cyclohexane acts by a non-specific mechanism in aquatic species.

3.2.1.3 Determination of Predicted No Effect Concentration (PNEC)Determination of PNEC_{aqua}

Results from acute tests only are available. As it is assumed that cyclohexane acts by a non-specific mechanism, a lower assessment factor $F = 100$ can be used on the most sensitive experimental result. Therefore the PNEC_{aqua} will be $900/100 = 9 \mu\text{g/l}$, based on the lowest available acute EC50 for fresh-water species (*Daphnia magna*).

Determination of PNEC_{microorganisms}

As effect data are available with specific aerobic bacterial populations (e.g., *Nitrosomas* sp.) as well as anaerobic bacteria, which would be exposed to the highest concentration if a denitrifying tank is present near the influent of the STP, a safety factor of 10 applied to the lowest EC50 seems to be sufficient. No EC50 was determined with *Tetrahymena elliotti* (Toxicity threshold = 24.2 mg/l), but the derived PNEC is probably protective of this species.

Therefore: $\text{PNEC}_{\text{microorganisms}} = 29/10 = 2.9 \text{ mg/l}$.

3.2.1.4 Sediment

As neither positive results from monitoring programmes on concentrations of cyclohexane in sediment nor experimental results with benthic organisms are available, a risk assessment cannot be performed for this compartment. As the chemical is only moderately hydrophobic, it can be assumed that the risk assessment for the sediment is covered by the risk assessment for surface water.

3.2.2 Atmosphere

Currier and Peoples (1954) studied the effects of cyclohexane vapour on barley and carrot plants. Barley plants were exposed 2 weeks after planting at the two-leaf stage and carrot plants were treated 63 to 95 days from seed. Plants were exposed for 0.5 to 2 hours and studied for injury immediately after exposure and 1, 14 and 28 days after the cessation of the exposure. At a cyclohexane concentration of 822.5 mg/m^3 , 10-25% of barley plants showed injury after 1 day and > 85% after 14 days. Carrot plants exposed to $1,233.7 \text{ mg/m}^3$ also showed 10-25% injury after 1 day but no more than 35% of plants showed injury after 28 days. The concentration of

cyclohexane causing 90-100% injury of plants after 1 hour was 1,545.4 mg/m³ for barley and 1,586 mg/m³ for carrot.

No PNEC can be derived from these results, as the exposure duration was very low.

As cyclohexane can undergo photochemical oxidation in the lower atmosphere, it may contribute to tropospheric ozone formation. A photochemical ozone creation potential (POCP) of 25 (as compared to 100 for ethylene, a chemical that is thought to be important in low-level ozone formation) is cited in Nielsen and Howe (1995).

3.2.3 Terrestrial compartment

Only a result from filter paper test with earthworms is available:

<i>Eisenia foetida</i>	48h LC50	> 1 mg/cm ²
(Roberts and Dorough, 1984)		

An extrapolation of this result to soil is not possible. Therefore, for an indicative risk assessment for the soil compartment, the equilibrium partitioning method will be used for an indicative PNEC:

$$PNEC_{soil} = K_{soil_water}/RHO_{soil} \cdot PNEC_{aqua} = 130 \mu\text{g}/\text{kg} \text{ (wet weight)}$$

or

$$PNEC_{soil} = K_{p_{soil}} \cdot PNEC_{aqua} = 147 \mu\text{g}/\text{kg} \text{ (dry weight)}$$

3.2.4 Secondary poisoning

Although cyclohexane presents a BCF in fish greater than 100, a risk assessment for secondary poisoning does not seem to be necessary, as it is not classified as “Toxic” or “Harmful” with at least R48 or R60-R64.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

Sewage treatment plants

An evaluation of the inhibition to microorganisms in STPs would seem opportune only for those situations where cyclohexane-containing wastewater is released to domestic treatment plants. Excluding therefore the production and transformation sites which usually have their own treatment plant; the only effluent concentration was determined for the use of cyclohexane as a solvent with $PEC_{\text{microorganisms}} = 232 \mu\text{g/l}$. With a $PNEC_{\text{microorganisms}}$ of $2,900 \mu\text{g/l}$, the PEC/PNEC ratio amounts to 0.08 and therefore a risk to microorganisms in STPs is not expected.

Conclusion (ii).

Surface waters

In **Table 3.18**, the comparison between PEC and the PNEC for all relevant exposure scenarios are presented. Only the local concentrations are taken into account, as the regional concentrations are negligible.

Table 3.18 PEC/PNEC for all relevant exposure scenarios

Scenario	PEC _{local} [$\mu\text{g/l}$]	PEC/PNEC
Production	< 1	< 0.1
Use as an intermediate (site-specific) ¹⁾	0.56	0.06
Use as a solvent for production processes in chemical industry	4.5	0.5
Formulation and use of adhesives and coatings	0	0

1) Highest release determined for 7 sites using cyclohexane as an intermediate

Conclusion (ii) applies to the use as a chemical intermediate, the use as a solvent for production processes in the chemical industry, as well as to the use in adhesives and coatings.

Sediment

As neither positive results from monitoring programmes on concentrations of cyclohexane in sediment nor experimental results with benthic organisms are available, a risk assessment cannot be performed for this compartment. As the chemical is only moderately hydrophobic, it can be assumed that the risk assessment for the sediment is covered by the risk assessment for surface water. The same conclusions as described above would therefore apply.

3.3.2 Atmosphere

The available test results on effects upon plants exposed via the gas phase do not allow the derivation of a PNEC and therefore a risk characterisation cannot be performed.

Due to the low atmospheric lifetime ($t_{1/2} = 52$ hours) as well as the absence of Cl and Br atoms, abiotic effects upon the atmosphere, like global warming and ozone depletion, are not to be expected from cyclohexane.

On the other hand a high potential for tropospheric ozone formation has been reported.

No conclusion can be drawn for possible atmospheric effects.

3.3.3 Terrestrial compartment

In **Table 3.19** the comparison between PEC and the PNEC for all relevant exposure scenarios are presented. Only the local concentrations are taken into account, as the regional concentrations are negligible.

Table 3.19 PEC/PNEC for all relevant exposure scenarios in soil

Scenario	PEC _{local soil} [$\mu\text{g}/\text{kg wwt}$]	PEC/PNEC
Production	0.9	0.007
Use as an intermediate	63	0.48
Use as a solvent for production processes in chemical industry	86	0.66
Formulation of adhesives and coatings	0.14	0.001
Use of adhesives and coatings	0.6	0.005

Conclusion (ii) applies to the use as a chemical intermediate, the use as a solvent for production processes in chemical industry as well as the use in coatings and adhesives.

3.3.4 Secondary poisoning

Although cyclohexane presents a BCF in fish greater than 100, a risk assessment for secondary poisoning does not seem to be necessary, as it is not classified as “Toxic” or “Harmful” with at least R48 or R60-R64.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

Cyclohexane is a liquid at standard temperature and pressure. Consequently, the inhalation, dermal and oral routes of exposure are relevant to any risk assessment. However, as cyclohexane is volatile, producing a colourless vapour, the inhalation route of exposure is the most relevant to both the working population and the general population.

4.1.1.1 Occupational exposure

Because of its physico-chemical properties, the overriding exposure route for cyclohexane is the inhalation route. There are no particular problems attached to measuring cyclohexane concentrations in workplace air. The sampling - analysis method (sampling on activated carbon, measurement by gas chromatography, and detection by flame ionisation) is sufficiently sensitive and specific for the concentrations presented in the literature and in the databases to be considered as reliable in this respect.

The different systems of limit values in force in each Member State are summarised in **Table 4.1**.

Table 4.1 Occupational Exposure Limits

Country	OEL - Occupational Exposure Limit (8-hour TWA) (ppm)	STEL - Short Term Exposure Limit (ppm)
US (for info.) (ACGIH-OSHA)	300 ^{a)}	- ^{a)}
Germany	200	-
United Kingdom	100	300
Netherlands	250	-
Sweden	300	370
Spain-Belgium-Italy-Greece	300	-
Austria-Denmark	300	-
France	300	375

* 1 ppm = 3.44 mg/m³ at standard temperature and pressure - 300 ppm correspond to ca. 1,030 mg/m³

a) The value of OEL in the US is currently being reviewed. An OEL value of 200 ppm is expected and a STEL of 400 ppm could also be applied.

4.1.1.1.1 Measured inhalation exposure data

Exposure during production (Scenario 1)

Cyclohexane is synthesised by hydrogenation of benzene. The process takes place in a closed system because of the flammability and toxicity of the substances used in the synthesis. The production units are in the open and thus benefit from a large amount of natural ventilation.

The exposure data provided by industry (**Tables 4.2** and **4.3**) summarise the measurements made by five European producers (Aromatics Producers Association, June 1995).

Table 4.2 Measured 8-hour Time Weighed Average exposure data (ppm)

Homogeneous exposure group (HEG)	Production					Filling operations	Filling + storage
	1994	1992-1994	june-dec. 94	1994		1992-1994	
Year or period	1994	1992-1994	june-dec. 94	1994		1992-1994	
Number of samples		114	153	20	2,756	18	281
Representative 8-hour TWA	< 3	0.19	< 0.5	2	0.75	0.22	0.64
Range (min-max)		0.05-1.61	<0.1-18.8	0.85-2.6	0-170	0.05-1.71	0-10
Comments (related exposure conditions, intermittent/continuous)			intermittent	continuous			
Respiratory protection worn (yes/no)	no	no	no	no		no	

Homogeneous exposure group (HEG)	Storage		Technical Dept	Labs		Maintenance
	1994	1992-1994		1992-1994		1992-1994
Year or period	1994	1992-1994		1992-1994		1992-1994
Number of samples		63	582	46	397	76
Representative 8-hour TWA	< 3	0.17	0.10	0.3	0.60	0.2
Range (min-max)		0.05-0.96	1-11	0.05-11.4	0-13	0.05-12.0
Comments (related exposure conditions, intermittent/continuous)						
Respiratory protection worn (yes/no)	no	no		no		no

Table 4.3 Short term or task measurements (ppm)

Homogeneous exposure group (HEG)	Filling operations	Transport (driver)
Year or period to which data below refers	1994	1994
Number of samples	25	25
Representative concentration and time (e.g. 10 ppm · 30 min)	31 · 30 min	36 · 30 min
Respiratory protection worn (yes/no)		

Workplaces were divided into the following activities: production, filling, storage, technical departments and laboratories, and maintenance operations.

The atmospheric concentrations determined from samples taken over 8 hours were on average less than 3 ppm (10.5 mg/m³), with maximum values not exceeding 20 ppm (70 mg/m³). These exposure levels were much lower than the corresponding limit values.

The atmospheric concentrations determined from short-duration samples taken during filling and transporting operations were approximately 30 ppm (105 mg/m³) for samples taken over a period of 30 minutes. Here again, the exposure levels were much lower than existing limit values.

Another producer indicates for production a value of ca. 20 ppm (70 mg/m³) (8-hour TWA) with no details of the number of samples.

Exposure during use as an intermediate or a solvent in the chemical industry (Scenario 2)

96% of the cyclohexane produced is used to synthesise caprolactam and adipic acid, which are intermediates in the production of 6 and 6.6 polyamides. Up to 2% is used as an auxiliary solvent in chemical production processes, mainly as a precipitating and extraction agent.

Synthesis of caprolactam and adipic acid are carried out in closed systems fed by pipes from storage tanks. Very few exposure data exist for these operations (in a European caprolactam unit: 4 measurements of less than 15 mg/m³, ca. 4 ppm).

Because process and working practices are likely to be similar during production and during use in the chemical industry, the atmospheric concentrations are assumed to be of the same order of magnitude for both scenarios.

Exposure during formulation and industrial use of cyclohexane containing products (Scenario 3)

Cyclohexane is used combined with other solvents:

- in neoprene-based adhesives for leather, fabric, wood, in the footwear industry, the clothing industry, the building industry, by craftsmen and by the general public,
- in natural rubber styrene-butadiene-styrene or styrene-butadiene-isoprene based adhesives used in the same types of activity,
- in industrial coatings and in printer inks,
- in varnishes for the interior coating of food packaging (application in development).

Exposure takes place during the formulation and use of products.

Literature data

Exposure data mainly concern the footwear industry, and to a lesser extent industrial paints and printing (during the use of products containing cyclohexane).

De Rosa et al. (1985) measured the cyclohexane concentration in 81 businesses in these three areas of activity. **Table 4.4** summarises the results of this study. Of 504 samples, 235 contained cyclohexane of which 223 were in the footwear industry.

Table 4.4 Summary of De Rosa et al. study on cyclohexane exposure

	Footwear industry	Paint	Printing
Number of samples	238	155	111
Number of samples containing cyclohexane	223	5	7
Range mg/m ³ (ppm)	10.5-1257 (3-365)	7-536 (2-156)	10.5-60 (3-17)
Number of samples with less than 525 mg/m ³ (ca. 150 ppm)	203	4	7
Number of samples with 525 to 1,050 mg/m ³ (ca. 150-300 ppm)	16	1	-
Number of samples with more than 1,050 mg/m ³ (ca. 300 ppm)	4	-	-

Bartolucci et al. (1986) measured the cyclohexane concentration at different workplaces in a shoe factory. 56 samples were taken (4 times over two hours) from 14 workers who were gluing or sewing. The results obtained by active sampling (pumping the atmosphere onto a porous support) ranged from 24 to 1,288 mg/m³ (7 to 374 ppm) of cyclohexane (average 301 mg/m³, 87 ppm).

Yasugi et al. (1994) measured the cyclohexane concentration at the workplaces of 33 workers applying adhesives containing only cyclohexane as the solvent or working in the vicinity of these particularly exposed workplaces. The geometric mean was 27 ppm (93 mg/m³), and the highest value was 274 ppm (943 mg/m³) (TWA).

Recently the Portuguese National Institute of Health carried out a study in 100 factories in Northern Portugal to assess solvent exposure in shoe manufacturing. Twenty-two organic solvents, including cyclohexane, were detected in the 1,161 air samples collected. Cyclohexane was not one of the most common pollutants: it was detected in less than 10% of selected factories. The results are only presented in graphs. They show that cyclohexane airborne concentrations (TWA) measured by personal sampling (n = 72) were always below the limit value of 1,000 mg/m³ (290 ppm) with a median value around 100 mg/m³ (29 ppm) and a maximum around 150 mg/m³ (43 ppm) (Mayan et al., 1999).

Finally, it should be noted that exposure levels at gasoline service stations were extremely low (0 to 0.1 ppm), which can be explained by the low concentrations of cyclohexane in gasoline (Kearney et al., 1986).

Information from databases

Data must be interpreted with care, as there are a number of possible sources of bias:

- none of the European Community countries has a systematic approach to sampling analysis. The data are collected for particular studies targeting a particular type of activity, region, or family of substances used. They may also give preferential treatment to high levels of exposure if they have been carried out as a result of a complaint from workers or their representatives,
- these data generally cover the results of personal sampling or of local air monitoring,
- in some cases (e.g. COLCHIC database in France) monitoring results are reported without checking the appropriateness of sampling/analysis methods or the sampling times.

The UK HSE data (1990) on cyclohexane are summarised in **Table 4.5**. Measurement of the cyclohexane concentration was not the main purpose in taking the samples. The activities shown appear to be relatively marginal. In all cases, exposure was very low and far lower than the UK Occupational Exposure Standard except in one special case which corresponds to a very marginal use.

Table 4.5 Data from the Occupational Hygiene Visits Register (OHVR) database of the Health and Safety Executive (HSE, UK, 1990)

Activity	Procedure used	Number of samples	Recorded levels (ppm)			TWA (ppm)		
			max	min	av	max	min	av
Diving suit manufacture	varied	30	4.1	ND	1.1	4.1	ND	1.1
Knitting machine manufacture	coating with paint	3	23	1.2	9.97	23	1	9
Air conditioning equipment	coating with paint	1		0.6			0.7	
Motor repair	lacquering	5	2.5	1.0	1.7			
Saw blades	lacquering	6	7.0	3.0	3.67	7.0	3.0	3.67
Museum	painting	1		3.1			2.0	
Police	finger printing	3	> 1,962	1,669	> 1,778	> 120	> 100	> 111

The COLCHIC system collects the trends and results of all the samples taken by the French National Research and Safety Institute (INRS) and the Occupational Risk Prevention departments of the Regional Health Insurance Funds. The data collected between 1988 and 1995 are presented in **Table 4.6**. Exposures were mostly lower than the most current Occupational Exposure Limit (300 ppm–1,032 mg/m³). The highest concentrations were mainly found during gluing operations carried out on wood, textiles, clothing and leather. Since the results did not take into account the sampling time, they may not necessarily reflect the whole shift.

Table 4.6 Data from the COLCHIC database of INRS (France), 1995

Occupational branch	Number of samples	Concentration range mg/m ³ (ppm)	Median mg/m ³ (ppm)	Number of samples with > 0- <100ppm	Number of samples with 100-300 ppm	Number of samples with > 300 ppm
Metal industry	233	0-2,594 (0-754)	8 (2.3)	168	15	12
Building and civil engineering	16	0-698 (0-203)	8.25 (2.4)	8	3	0
Woodworking industry	65	0-3,284 (0-954)	7 (2)	37	0	8
Chemicals	96	0-537 (0-156)	3 (1)	84	6	0
Rubber, paper, cardboard	78	0-394 (0-114)	8 (2.3)	70	1	0
Books	13	0-6 (0-2)	0	3	0	0
Textiles	11	17-423 (5-123)	110 (32)	10	1	0
Clothing	14	0-392 (0-114)	169.5 (49)	7	1	0
Leather, furs	66	0-486 (0-140)	67.25 (19)	58	5	0
Water, gas, electricity	1	5 (1.4)		1	0	0
Non-food trade	10	0-1	0	1	0	0
Not otherwise classified	22	0-64 (0-18)	2 (< 1)	16	0	0

The information from the databases shows that exposure levels during use in industrial sectors are often lower than the limit value; sometimes this exposure approaches the limit value of 300 ppm (1,032 mg/m³) (current value in most countries) and in some cases it may attain

954 ppm (3,282 mg/m³) when working conditions are very bad and when workplace regulations are not applied.

Exposure during use of cyclohexane containing products in craft industries (Scenario 4)

Exposure of craftsmen using the types of adhesives described above is likely to be relatively high. This group includes shoe repairers, carpet layers, mattress makers, decorators, cabinet makers, etc. While the quantities of product used may be low, good working practices may often not be followed (small or badly ventilated areas, containers not closed after use, etc.).

In a study carried out by TNO in the Netherlands, cyclohexane exposure levels of carpet layers using-solvent based adhesives were measured on days with relatively high usage of adhesives. Ventilation was natural, sometimes windows were opened. Personal was carried out on 37 subjects, cyclohexane was detected in the range from 3 ppm to 159 ppm (10-547 mg/m³) with a geometric mean concentration of 15 ppm (52 mg/m³) and a 90-percentile concentration of 85 ppm (292 mg/m³). No personal protective equipments were used (TNO, 1996). There are no other measured data available.

4.1.1.1.2 Inhalation and dermal exposure data derived from modelling

The Estimation and Assessment of Substance Exposure (EASE) model, developed by the EU, has been used to predict inhalation and dermal occupational exposure to cyclohexane.

Production (Scenario 1) and use as an intermediate or as a solvent in the chemical industry (Scenario 2)

These processes involve the use of closed system and batch production methods. The exposure is estimated to be low (between 0 and 0.1 ppm) (0.34 mg/m³) if the level of control is full containment. Some higher exposures are likely to occur in some operations (tanker loading, cleaning, sampling, etc.), leading to an estimate of 10-50 ppm (34-172 mg/m³) (moderate volatility, non-dispersive use with local exhaust ventilation).

It can be considered that dermal exposure only occurs during some operations with a EASE estimation of 0-0.1 mg/cm²/day (non dispersive use and incidental contact). This value does not take into account the use of personal protective equipments or the evaporation of cyclohexane from the skin.

Formulation and industrial use of cyclohexane containing products (Scenario 3)

Cyclohexane is generally used with local exhaust ventilation or dilution ventilation. The exposure is therefore supposed to be 10-50 ppm (34-172 mg/m³) or 100-200 ppm (344-688 mg/m³) (moderate volatility, non-dispersive use).

For dermal exposure, the EASE model gives the following range: 0.1-1 mg/cm²/day (non dispersive use and intermittent contact). The typical cyclohexane content in products is 30%, which leads to a dermal exposure for this scenario of 0.03-0.3 mg/cm²/day. This value does not take into account the use of personal protective equipments or the evaporation of cyclohexane from the skin.

Use of cyclohexane containing products among craftsmen (Scenario 4)

Artisans generally work without any collective protection device or personal protective equipment (wide dispersive use, direct handling): exposure is predicted to be as high as 500-1,000 ppm (1,720–3,440 mg/m³). Where dilution ventilation systems are used, exposure is estimated to be in the range of 200-500 ppm (688–1,720 mg/m³).

Considering the dermal exposure, the EASE model gives the following range: 5-15 mg/cm²/day (dispersive use and extensive contact). Given that the products used contain a maximum of 30% cyclohexane, the estimated dermal exposure is 1.5-4.5 mg/cm²/day. This value does not take into account the evaporation of cyclohexane from the skin or the use of personal protective equipment. Gloves are misused or are used for excessively long periods of time (with possibility to have cyclohexane inside the gloves unable to evaporate).

4.1.1.1.1 Conclusion of occupational exposure

Table 4.7 Conclusion of occupational exposure assessment

Scenario	Estimated inhalation exposure ppm (mg/m ³) ^{a)}		Estimated skin exposure (mg/cm ² /day) ^{b)}
	Long term (8-hour TWA)	Short term	
1-Production	20 (69)	30 (103)	0-0.1
2-Intermediate	20 (69)	30 (103)	0-0.1
3-Formulation and industrial use of products	300 (1,032)		0.03-0.3
4-Use of products in craft industries	200-1,000 (688–3,440)		1.5-4.5

a) Values derived from measured data and expert judgement for Scenarios 1, 2 and 3 and derived from EASE model for Scenario 4

b) Values derived from EASE dermal exposure model

4.1.1.2 Consumer exposure

No quantitative data could be obtained for the evaluation of consumer exposure, neither from the chemical industry nor through the review of the literature.

Most of the cyclohexane produced is used in the synthesis of other substances within the chemical industry and does not reach consumers. However, consumer exposure can occur where cyclohexane is used as a solvent in adhesives, paints, inks, varnishes, floor polishes, waxes and possibly in other household products. It is known that some of these preparations may contain a high percentage of cyclohexane: for instance, adhesives with 10-30% cyclohexane may be delivered in large containers.

According to data provided by the French, Swedish and Danish product registers, adhesives containing cyclohexane is by far the main consumer use and this assessment will be focused on this use category. Adhesives are used for carpet laying but may also be used for a wide variety of application. The lack of information about the actual application makes it difficult to define the exposure assessment and to propose different scenarios.

Only the scenario carpet laying for which the highest exposure is expected will be considered.

Exposure from carpet adhesives

Consumer exposure during carpet laying is expected to be similar to the exposure of professional carpet layers who often work in private homes. However, for consumers the frequency will be very low and an acute time scale is considered appropriate.

Inhalation exposure

In a study performed by TNO in the Netherlands, the cyclohexane exposure levels of professional carpet layers using solvent-based adhesives were measured on days with relatively high usage of adhesives. Ventilation was natural, sometimes windows were opened. 37 personal samplings were performed, cyclohexane being detected in the range from 3 ppm to 159 ppm (10-547 mg/m³) with a geometric mean concentration of 15 ppm (52 mg/m³) and a 90-percentile concentration of 85 ppm (292 mg/m³) (TNO, 1996). There are no other measured data available.

Exposure as high as 500-1,000 ppm (1,720–3,440 mg/m³) can be predicted using the EASE model (wide dispersive use, direct handling). Where dilution ventilation systems are used, the exposure is estimated to be in the range of 200-500 ppm (688–1,720 mg/m³).

In conclusion, the upper range of the EASE estimates (1,000 ppm equivalent to 3,440 mg/m³) will be used as a reasonable worst case for acute consumer exposure during carpet laying.

Dermal exposure

Using the EASE model, dermal exposure is estimated in the following range: 5-15 mg/cm²/day (dispersive use and extensive contact). Given that the products used contain a maximum of 30% cyclohexane, the estimated dermal exposure is 1.5-4.5 mg/cm²/day.

4.1.1.3 Humans exposed via the environment

The estimation of indirect human exposure via the environment is presented in Annex 1. The total daily intake based on the local as well as regional environmental concentrations due to the different uses are presented in **Table 4.8**. As the exposure is mostly due to air exposure, the average atmospheric concentrations as well as the contribution of the atmospheric concentrations to the overall daily dose are reported as well.

Table 4.8 Total daily intake due to local and regional environmental exposures

Scenario	DOSEtotal (mg/kg bw/day)	Average air concentration (µg/m ³)	Fraction of total dose through intake of air
Production	0.037	175	0.99
Use as an intermediate	0.082	381	0.99
Use as a solvent for production processes in the chemical industry	0.115	521	0.97
Formulation of adhesives and coatings	0.006	27	0.99
Use of adhesives and coatings	0.026	123	0.99
Regional exposure	9.10 ⁻⁵	0.35	0.87

The highest indirect exposure is estimated for the use as a solvent for production processes in the chemical industry.

In addition to the indirect exposure due to the industrial use of cyclohexane, humans are exposed through car exhausts. As seen above, the measured concentrations of cyclohexane in the vicinity of busy roads are approximately 1-10 $\mu\text{g}/\text{m}^3$. Assuming a respiration rate of 20 m^3/d , a bio-available fraction of 0.75 for inhalation and an average body weight of 70 kg, a daily intake of 0.00021-0.0021 mg/kg bw/d can be estimated.

4.1.1.4 Combined exposure

The combined exposure is mainly the occupational exposure, especially in small industries when considering the chronic health hazards and the exposure of consumers, when considering the acute effects, through its use as solvent in glues and household products.

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

Good Laboratory Practices (GLP) statements were checked for all the studies. When available, this information has been provided. Otherwise, no comment is made.

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Oral

The toxicokinetics of cyclohexane has been studied in both rats and rabbits.

Studies were performed on Fisher 344 rats to determine the absorption, distribution, metabolism and excretion of [^{14}C] cyclohexane (Research Triangle Institute, 1984). When dosed intravenously 54% of the dose was exhaled within 24 hours of administration. After 72 hours, 83% of the dose had been exhaled and 14% was excreted in the urine. A similar pattern was observed after oral administration (100-2,000 mg/kg). In this case, the higher the dose of cyclohexane, the higher the proportion of cyclohexane excreted via the lung (63-78%). The other major route of excretion was via the urine (12-29%). No significant excretion was found via faeces. This implies that there is rapid absorption of cyclohexane from the gastro-intestinal tract after oral administration.

In this study, cyclohexane accounted for the majority (93-99%) of the radiolabel exhaled in breath but less than 0.1% of that in urine. Small amounts of cyclohexanone and cyclohexanol were exhaled in breath or excreted in urine. The other metabolites were not ascertained but they eluted in the migration region of conjugates (in HPLC).

[^{14}C] from radio labelled cyclohexane dosed orally was present in adipose tissue at 16 times the concentration found in blood, even at time intervals of 6, 24 or 72 hours after dosing. Consequently the half-life of [^{14}C] in plasma and tissues (including adipose) was found to be about 10-15 hours. Small amounts of cyclohexane and cyclohexanol were present in all tissues.

In a rabbit study where animals were given [^{14}C] cyclohexane at doses between 100 and 390 mg/kg via the oral route, expired air was monitored for 2 days after dosing, and urine and faeces for 2-6 days (Elliot et al., 1959). In this case, the proportion excreted in exhaled air and urine was similar (35-47% and 46-55%, respectively), but again minimal amounts of [^{14}C] were detected in the faeces (0-0.2%). A small amount of radioactivity was detected in tissues after 2-6 days. In the urine, glucuronides of cyclohexanol and cyclohexanone were the main metabolites detected with some glucuronides of cyclohexane-1,2-diol. The authors suggest however that the cyclohexane conjugate may have been an artefact of the analysis.

As in the rat the exhaled [^{14}C] was present mainly as unchanged cyclohexane (25-38% of dose) with some [^{14}C] O_2 .

When reduced doses were given, (0.3 mg/kg), the principal route of excretion was the urine (87%) in 4 days. 5.5% was excreted via exhaled air as [^{14}C] O_2 , and no unchanged cyclohexane was detected.

4.1.2.1.2 Dermal

Dermal absorption of cyclohexane has been assessed by RTI (1996). Three sets of two studies were performed on Fischer F344 rats. The first study in each set examined the excretion routes and rates for [¹⁴C] cyclohexane and its metabolites, and measured the body burden of total radiolabel at the end of the study. The second study in each set examined blood concentrations of cyclohexane and its metabolites (total radiolabel) as a function of time. In the first set of studies, the animals (6/sex/group) were exposed dermally (occluded) to [¹⁴C] cyclohexane for 6 hours at a concentration of 1 mg/cm² (primarily as cyclohexane vapour). In the second set, the animals (6/sex/group) were exposed to 100 mg/cm² (primarily as liquid cyclohexane) in the same experimental conditions. The third set was designed to provide baseline information, a 10 mg/kg dose of cyclohexane was administered intravenously to groups of 5 animals (it was anticipated that this dose would result in blood levels of radioactivity similar to those produced in the 1 mg/cm² dermal dose).

For the 1 mg/cm² dermal dose of cyclohexane, the average absorption rates were 0.06 and 0.1 mg/cm² of exposed skin/h for male and female rats, respectively. In this case, this corresponded approximately to a 40-60% range of absorption of the applied dose. Exposure at this dose level was primarily to cyclohexane vapours. For the 100 mg/cm² dose, there were no overt differences between males and females. The average absorption rate was 0.65 mg/cm² of exposed skin/h and the absorbed dose was about 4% of the total applied dose. At this dose, exposure was primarily to liquid cyclohexane. Metabolite nature was not determined. Excretion of cyclohexane was rapid after dermal or intravenous administration. There were no signs of accumulation in the body 72 hours after exposure, less than 0.1% remaining in the carcass after 100 mg/cm² exposure and less than 0.4% for the 1 mg/cm² exposure. The major route of excretion after both intravenous and dermal application was pulmonary excretion. Expired breath accounted for ca. 70% of excreted radiolabel following intravenous administration and for ca. 78% and 57% following dermal exposures to 1 and 100 mg/cm², respectively. Urinary excretion of the radiolabel accounted for ca. 29% after intravenous injection and for ca. 20 and 40% after 1 and 100 mg/cm² dermal exposure, respectively.

According to the determined values, it would appear that there were differences between males and females for absorption of small doses of cyclohexane, but in terms of individual data, one female and one male had abnormal values for absorption. Studies on the dermal absorption of organic chemical vapours (Mc Dougal et al., 1990) have suggested that there may be considerable variations between individual rats. Due to the small group size, it is not possible to determine with certitude if these data should be taken into consideration or not, so, for risk assessment purpose, the most conservative value is chosen.

In a comparison of four solvents, including cyclohexane, for their ability to induce inflammation, Iyadomi et al. (1998) glued chambers to the abdominal skin of hairless rats (exposed skin surface 3.14 cm²) and introduced 1 ml of pure solvent. Hairless rats were used since there is no need to shave them, thus preventing damage to the stratum corneum, which is the most important barrier in the skin. The blood solvent concentration was measured at 4 time points (10, 30, 60 and 240 min, with two animals at each time point). The cyclohexane concentration increased for 1 h (maximum value ca. 0.24 mmol/l) and then decreased until the end of the exposure. Toluene and cyclohexane caused rapid histological changes such as perinuclear oedema, karyopycnosis, spongiosis, intra-epidermal vesicles and marked epidermal-dermal separation. Skin modifications following the application of liquid cyclohexane have been known for several decades (Brown and Box, 1971). This paper confirms that cyclohexane is rapidly absorbed through rat skin and has marked skin irritation properties. Data are insufficient to estimate a

penetration rate. They also differ qualitatively from those of the RTI (1996) study, and may be the result of cyclohexane losses through leaks or evaporation, or because cyclohexane alone - and not total radiolabel, which can include metabolites - is analysed by gas chromatography.

4.1.2.1.3 Inhalation

Studies in animals

A study performed by TNO (1998a) attempted to determine the kinetic constants V_{max} and K_m for cyclohexane metabolism in rats. These values were subsequently compared in a physiologically based pharmacokinetic (PbPk) simulation model in cross species extrapolation. This study is not used in the risk characterisation and is given for information in Annex 1.

Studies in humans

A number of papers have been published on the absorption, metabolism and excretion of cyclohexane, the majority of which are centred around exposures to cyclohexane used as a solvent in the Italian shoe industry during the late 1970's.

In a study on the lung uptake of cyclohexane on five workers and three volunteers, Mutti et al. (1981) found that about 23% of inhaled cyclohexane was taken up by the lungs. In the case of large amounts, 40% of the dose was excreted unchanged in the air and another 10% exhaled as carbon dioxide. When doses were much lower, only 10% of the absorbed cyclohexane was exhaled unchanged and 5% metabolised as carbon dioxide. Urinary excretion of metabolites (mainly cyclohexanol) was found to be only 1% of the absorbed dose.

In a similar study Perbellini and Brugnone (1980) determined cyclohexane in the environmental air, alveolar air, and the blood and urine of shoe factory workers. The environmental concentrations ranged from 17 to 2,484 mg/m³. Alveolar concentrations of cyclohexane in 59 samples from 22 workers clearly mirrored these, with concentrations from 16 to 1,929 mg/m³. The mean alveolar concentration corresponded to 78% of environmental cyclohexane concentrations. Blood cyclohexane, 4 hours after exposure, ranged from 29 to 367 µg/l, which in turn correlates to 53-78% of alveolar concentrations. In this study, urinary cyclohexanol corresponded to 0.1-0.2% of the cyclohexane absorbed. Although low, the excretion of cyclohexane metabolites was correlated with cyclohexane blood levels.

The same authors measured and identified the metabolites of cyclohexane in the urine. As cyclohexanol was found as the main metabolite, this suggested that cyclohexane is mainly metabolised via hydroxylation in man and that cyclohexanol could potentially be used as a marker for exposure (Perbellini et al., 1980; 1981).

Exposure to nine solvents was studied by Ghittori et al. (1987) on 659 male subjects working in plastic boat, chemical, plastic button, paint and shoes factories. A group of 43 workers was monitored specifically for cyclohexane. Urine was collected after a 4-hour exposure period at the beginning of the work shift of the urinary solvent concentration (C_u) was determined by gas chromatography. The weighted environmental concentration in the breathing zone (C_i) over the 4 hours of exposure was measured using a personal passive dosimeter.

Overall, the C_u values showed a linear relationship with the corresponding C_i values. The regression equation, the correlation coefficient, the 4-hour exposure C_u value (mean)

corresponding to TLV-TWA and the proposed BEEL (Biological Equivalent Exposure Limits) in µg/L of cyclohexane are summarised in **Table 4.9**.

Table 4.9 Relationship between urinary solvent concentration and solvent exposure

Regression equation between urinary solvent concentration and solvent exposure	Correlation coefficient	Urinary solvent concentration (corresponding to TVL-TWA)	Proposed BEEL
$Y=0.05X + 8.26$	0.89	65 µg/L	57 µg/L

In a survey conducted by Yasugi et al. (1994) on 33 women who either applied glue (with cyclohexane as an "almost exclusive solvent component") or worked in the vicinity of glue application, the geometric mean and the highest cyclohexane concentration observed in air were 27 and 274 ppm, respectively (93 and 943 mg/m³). Quantitative estimates at the end of shift suggested that only a minute portion (<1%) of cyclohexane is excreted in the urine as cyclohexanol (almost exclusively as a glucuronide). In this study the biological half time was estimated as 5 hours.

Mraz et al. (1998) conducted a series of studies on humans to compare the metabolic pathway of inhaled cyclohexane, cyclohexanol and cyclohexanone at doses close to OEL (Occupational Exposure Limit) values. The mechanism of elimination of the biological markers was also studied (cyclohexanol, 1,2-cyclohexanediol and 1,4-cyclohexanediol) by means of a test assessing the binding to the blood proteins and by oral administration of the two major metabolites recovered in man: 1,2- and 1,4-cyclohexanediol.

Four men and four women (31-55 years old) were exposed in a closed exposure chamber to cyclohexane and cyclohexanol at doses of 294 ± 10 ppm ($1,010 \pm 35$ mg/m³) and 69 ± 0.6 ppm (236 ± 2 mg/m³), respectively for an eight-hour period. Data from another study (Mraz et al., 1994a) using cyclohexanone was included in order to compare the three substances. The minute respiratory volume and the mean retention in the respiratory tract were calculated. Oral administration of cyclohexanediols pooled and dissolved in water was performed on volunteers at doses of 2 mmol each (1,2- and 1,4-cyclohexanediol) corresponding to 232.2 mg. Urine was collected for 72 hours and analysed by gas chromatography to determine cyclohexanol and cyclohexanediol concentrations. Acidic hydrolysis was also performed to determine the ratio of conjugated/unconjugated proportion of metabolite. Cis and trans isomers of cyclohexanediols were not resolved but it is known that trans isomers predominate (Flek, 1989; Mills, 1990). For the binding study, human plasma (20 ml) was placed in a dialysis casing immersed in 0.15 M phosphate buffer (30 ml) containing cyclohexanediol (2 or 10 µmol of each isomer). One system was constructed without cyclohexanol and served as negative control. The systems were allowed to stand at 37° for 18 hours, then the outer buffer was analysed for cyclohexanediols.

As demonstrated in previous studies (Mraz et al., 1994b), cyclohexane was metabolised in cyclohexanol, 1,2-cyclohexanediol and 1,4-cyclohexanediol (the two later being in the majority: only 1% of the absorbed dose was excreted as cyclohexanol). The cyclohexanol peak was identified just after exposure and the half-life of elimination was calculated as 1.5 hours. The excretion of cyclohexanediols was maximum after a few hours of exposure and elimination half-lives were 17 ± 5.2 and 16.1 ± 3.9 for 1,2- and 1,4-cyclohexanediol, respectively; excretion curves of the metabolites were similar regardless of whether the subjects had been exposed to CH (cyclohexane), CH-ol (cyclohexanol) or CH-one (cyclohexanone). The ratio of metabolic yields of 1,4- and 1,2- isomers was independent of the parent compound. There were no differences between men and women with regard to the metabolic yields and elimination

half-lives of the metabolites. CH-one was not detected in urine with CH-ol, CH and CH-one exposure. However, with a more sensitive method, Yasugi et al. (1994) demonstrated that CHone was present at very low concentration following an occupational exposure to cyclohexane.

Half-lives of the metabolites indicated that their elimination was quite slow and that it was the common rate-limiting step in the overall elimination of the three compounds. This slow elimination could be due to a specific non-covalent binding to blood proteins of these compounds. In order to confirm this hypothesis, a binding test was performed. The results demonstrated that only negligible binding of CH-diols to plasma was expected. According to the authors, this was quite surprising since the elimination of polar conjugates such as glucuronides (1,2-diol) is expected to be significantly faster than unconjugate compounds (1,4-diol).

In the ingestion study, the excretion peak was attained within 4 hours. Elimination half-lives were similar to those described in other studies (metabolism products of cyclohexane, cyclohexanol and cyclohexanone). 57% and 76% of the delivered dose of 1,2-diol and 1,4-diol, respectively were excreted over a period of 72 hours. Given the half-lives of these compounds, the total recovery did not exceed 60% and 80%, respectively, the author's conclusions for this finding being that CH-diols undergo further metabolism. 1,2-diol appeared in urine as glucuronide (> 95%) whereas 1,4-diol was excreted unconjugated. It has been postulated that humans differ considerably from animals in the ratio of CH-ol oxidation rate (Mraz, 1994).

Perico et al. (1999) compared the kinetic profile of 1,2- and 1,4-cyclohexanediol in urine suggested by the PBPK model with the results obtained in workers. Individual exposure was measured in 156 workers (in 19 shoe and leather factories) by measurements of 1,2- and 1,4-cyclohexanediol in urine. Samples were collected on different days during a working week: 29 workers provided urine samples on Monday before working, 47 provided urine samples on Thursday at the end of the working shift and Friday morning, and 86 others provided urine only at the end of the work shift, on Monday or Thursday. The PBPK model was based on a model previously described by Perbellini et al. (1988-1990). K_m , V_m and half-life of 1,2- and 1,4-cyclohexanediol were assumed to be those defined in previous metabolism studies.

Individual exposures ranged from 2 to 179 ppm (7 to 617 mg/m³ (with a mean of 60 mg/m³)). Urinary concentrations of 1,2-cyclohexanediol were 3.1-7.6-13.2 and 6.3 mg/g creatine on Monday (pre- and post-shift) - Thursday (post) and Friday (pre), respectively. The corresponding values recorded for 1,4-cyclohexanediol were 2.8-5.1-7.8-3.7 mg/g creatine. A close correlation was found between environmental exposure to cyclohexane and post shift urinary 1,2-cyclohexanediol on Monday. Data collected on Thursday and Friday showed only a poor correlation to exposure. For 1,4-cyclohexanediol, the concentrations found in samples collected on both Monday and Friday morning were not statistically different from the non exposed people. Only data collected on Monday and Thursday at the end of the work shift showed concentrations statistically higher than the others. Both metabolites have an urinary elimination half-life of 16 and 18 hours, respectively for 1,2-cyclohexanediol and 1,4-cyclohexanediol (also determined by Mraz et al., 1994) and accumulate during the working week. The comparison with the PBPK model gave similar results.

In another study, Mraz et al. (1999) studied the effect on cyclohexanol and cyclohexanediols excretion during cyclohexane, cyclohexanol and cyclohexanone exposure concurrently with ethanol administration. For cyclohexane, 5 volunteers were submitted to a 300 ppm (1,032 mg/m³) 8-hour exposure and given 4-14g of ethanol at given times during the experiment. Urine was collected for 72 hours (18 samples/volunteer). Cyclohexanol and

cyclohexanediol concentrations were determined by gas chromatography. 8 volunteers from a previous study served as negative controls (Mraz, 1998). The only difference between ethanol-treated subjects and controls was an increase in the concentration of cyclohexanol in the urine (6-fold higher for cyclohexane and cyclohexanol and 11-fold higher for cyclohexanone). There was no difference in the concentrations of 1,2-cyclohexanediol and 1,4-cyclohexanediol in the urine of ethanol-treated subjects and controls. The authors concluded that cyclohexanediols could be better indicators than cyclohexanol for monitoring cyclohexane, cyclohexanone and cyclohexanol exposures.

In a Research Triangle Institute study (1980), samples of milk from mothers were collected from different towns and different states of the US and analysed for volatile (purgeables) and semi-volatile (extractable) organics (including cyclohexane) using glass capillary gas chromatography/mass spectrometry/computer. The limits of detection were about 20-100 ng/ml milk. Of the eight samples tested, cyclohexane was present in six confirming the ability of this compound to be excreted via maternal milk.

4.1.2.1.4 Additional data: enzymatic induction

In a series of studies, Espinosa-Aguirre et al. (1996; 1997) attempted to demonstrate the enzymatic induction of cytochromes P450 (CYP 450) due to cyclohexanol treatment in rats and which isozyme is involved. It had previously been demonstrated (Espinosa-Aguirre et al., 1993) that cyclohexanol had anti-mutagenic properties *in vivo* and *in vitro* when administered with NDMA and NDEA. This property was apparently due to the capacity of cyclohexanol to inhibit the metabolism of nitrosamines by a competitive mechanism. Concurrently with this mechanism, cyclohexanol was also able to induce CYP 450. The experimental procedure to prove these findings is based on a pre-incubation mutation test on *Salmonella typhimurium* TA100 performed with metabolic activation (S9) pre-treated or not with cyclohexanol. S9 fractions were prepared from cyclohexanol induced male Wistar rats (2.5% *ad libitum* in the drinking water for 5 days). In order to characterise which isozyme was involved, immunoblot analysis employing monoclonal antibodies was performed. The presence of CYP1A1/A2 - CYP2B1/2B2 and CYP2E1 was investigated.

It was demonstrated that there was an induction of CYP2E1 and CYP2B1/B2 but not of CYP1A1/A2.

4.1.2.1.5 Summary of toxicokinetics, metabolism and distribution

For animals, by oral and inhalation route, cyclohexane is almost completely absorbed. For the dermal route, an absorption of 50% can be estimated for low concentrations (vapour phase) and only 5% of absorption for direct contact with the liquid.

Cyclohexane is readily distributed to all tissues with a preference to adipose tissues (concentrations of cyclohexane were found to be 16 fold higher in adipose tissues than in blood) but with no evidence of accumulation. Although not assessed in specific studies, due to the low molecular weight, the lipophilic properties of cyclohexane and the nervous symptomatology noted in all the studies, cyclohexane is probably able to cross the blood brain-barrier. Due to the low molecular weight and to the SAR with other substances, cyclohexane is assumed to be able to cross the placental barrier.

Cyclohexane is rapidly metabolised in the liver. Successive hydroxylation's and oxidation lead to the formation of various quantities of cyclohexanol, cyclohexanone, 1,2-cyclohexanediol and 1,4-cyclohexanediol, depending on the dose administered and the species considered. Excretion of glucurono-conjugate metabolites is also possible and depends on species.

In humans, the main metabolic pathway leads to the formation of a majority of 1,2- and 1,4-cyclohexanediols excreted unchanged for 1,4-cyclohexanediol and in glucuronide form for 1,2-cyclohexanediol. The ratio of 1,2-/1,4- cyclohexanediols is independent of the dose and of the gender. The major difference between humans and animals is the formation of cyclohexanol (in majority in animals) and cyclohexanediols (in majority in humans). In all species, the concentration of cyclohexanone formed is very limited.

In rats, an induction of CYP 2E1 and CYP 2B1/B2 was demonstrated and there were no effects on CYP 1A1/A2.

Elimination via the lungs was the major route of excretion (higher with increasing doses of cyclohexane) as unchanged cyclohexane or CO₂.

Elimination of the metabolites was quite slow in the urine (half lives of elimination of 16 and 18 hours for 1,2- and 1,4- cyclohexanediols, respectively in humans) and accumulated as the week progressed. This step is the rate-limiting step of the overall elimination of cyclohexane in humans. The biological half-lives were estimated to be about 10-15 hours in rats by oral route and to be 5 hours in human by inhalation.

It has been shown that an excretion via the milk is possible.

4.1.2.2 Acute toxicity

4.1.2.2.1 Oral exposure

Oral LD50 values of > 5,000 mg/kg, 29,800 mg/kg and 8,000-39,000 mg/kg have been reported for cyclohexane in rats (Phillips Petroleum Company, 1982a; Deichmann and Le Blanc, 1943; Kimura et al., 1971, respectively).

Kimura et al. showed that the oral LD50 was dependent on the age of the animal. The oral LD50 in a 14-day old rat, a young adult rat and an older rat was 8.0, 39.0 and 16.5 ml/kg, respectively (6,240, 30,420 and 12,870 mg/kg, respectively). Symptoms included depressive effect on the central nervous system, salivation and soft faeces.

In another study, the lowest lethal dose for rabbits by oral administration was found to be 6 g/kg. Symptoms included severe diarrhoea, great loss of weight and increased respiration rate, but the authors found no evidence of acute involvement of the central nervous system (narcosis or convulsions) (Treon et al., 1943a).

4.1.2.2.2 Dermal exposure

An acute dermal toxicity test is available on cyclohexane in rabbits (Phillips Petroleum Company, 1982c) with the LD50 estimated to be greater than 2,000 mg/kg. No deaths or systemic symptoms were observed, a slight erythema and oedema were noted in a few animals.

4.1.2.2.3 Inhalation exposure

Acute inhalation exposure of rabbits to cyclohexane vapour during 1 hour produced effects on the central nervous system, with exposure-related observations including convulsions, tremors, hyperactivity, rapid respiration, cyanosis and diarrhoea; all the animals exposed to 26,000 ppm (89.6 mg/l) died (Treon et al., 1943b). The 4-hour exposure LC50 on rats was > 9,500 ppm (32.88 mg/l); no death occurred at this concentration; exposure-related symptoms noted during the exposure included tremors, hyperactivity, rapid respiration and also hypo activity (Phillips Petroleum Company, 1982b).

Acute neurotoxicity

Studies in animals

The neurotoxicity of acute exposure to cyclohexane was studied with schedule-controlled operant behaviour (SCOB) (Haskell, 1996c). Male rats (10/group) were food-limited and trained to press a lever in order to obtain food. They were progressively introduced to a multiple fixed ratio fixed interval (FR20-FI120 seconds) schedule of reinforcement. The rats were trained 5 times a week for 5 to 6 weeks prior to cyclohexane exposure. Baseline response was stable before exposures. Four groups were exposed to 0-500-2,000 and 7,000 ppm of cyclohexane (0–1,720–6,880 and 24,080 mg/m³) (whole body in an exposure chamber). The operant test session began about 30 minutes after the 6-hour exposure. The parameters measured were:

- fixed ratio response rate,
- fixed ratio pause duration,
- fixed interval response rate,
- fixed interval index of curvature.

This study was combined with two positive control studies, performed according to the same experimental procedure, one study with a chlorpromazine treatment the other with an amphetamine treatment. These studies were performed according to EPA and OECD GLP.

The only changes observed was a slight decrease (11%) in the fixed ratio rate for the 7,000 ppm group 30 minutes after exposure (this change could be due to a mild-sedative effect). No more changes were noticed for up to two weeks following exposure. For this study, a NOAEL of 2,000 ppm (6,880 mg/m³) can be assumed for changes in SCOB after acute exposure to cyclohexane.

A study performed by TNO in 1998 (TNO, 1998b) attempted to evaluate the behavioural effects of exposure to cyclohexane in animals and to determine internal levels of exposure at which effect occur. This study was part of wide-ranging TNO program on inhaled solvents to establish the validity of a physiologically based pharmacokinetic (PbPk) model in rats and humans for predicting human neurobehavioral effects on the basis of animal neurotoxicity data.

This study comprised three experiments:

- experiment I: rats were submitted to an inhalation exposure to cyclohexane and the effects evaluated on standardised observational measurements and motor activity,
- experiment II: speed and accuracy of the animals were evaluated in a discrete-trial visual discrimination task,
- experiment III: blood and brain concentrations of cyclohexane were determined in separate groups of rats.

Groups of WAG/Rij Crl BR male rats (32 in experiment I, 36 in experiment II and 95 in experiment III) were exposed to cyclohexane (purity > 99%) at different concentrations for one or three 8-hour exposures. Three concentrations were tested: 400 ppm (1,400 mg/m³)-2,000 ppm (8,000 mg/m³) and 8,000 ppm (28,000 mg/m³). For experiment III (kinetic experiment), groups of animals were sacrificed at 2 hours, 4 hours or immediately following the 8-hour exposure period (one or three exposures) and at different times following the 8-hour exposure period: 0.5 h - 1 h-2 h-4 h and 8 h. Both blood and brain samples were collected at each sampling time.

For experiment I, following parameters were evaluated:

- neuromuscular: gait, forelimb and hind limb grip strength, landing foot splay,
- sensimotor: response to tail pinch, click, touch and approach of a visual object,
- convulsive: clonic and tonic movements,
- excitability: arousal,
- activity: motor activity.

FOB was performed 6 days before the beginning of the study and immediately after the first and the third observation.

For experiment II, water-limited animals were trained for four weeks in the discrete-trial two-choice discrimination task (with water reinforcement) in order to stabilise baseline responses. Test sessions consisted of 100 trials or 60 min whichever came first. Trials were signalled by the illumination of either the left or the right stimulus light (S+) and the rat's task was to depress the lever under the illuminated light in order to obtain the water reward. If the rat pressed the correct lever (S+ response) the stimulus light was extinguished and a water reward (SR+) was delivered. If the initial response during a trial was on the incorrect lever (S- response) the rat was allowed to correct its mistake by pressing the lever under the illuminated stimulus light. A given trial remained in effect until the correct lever was pressed. Trials were separated by an inter-trial interval (ITI) of 10 s. A response during the ITI reset the ITI timer and the rat was required to wait a further 10 min before the initiation of the following trial. On days of exposure, the rats were tested immediately following the end of the exposure period.

The correctness of the initial response in each trial was recorded for each rat. If the initial trial response was correct (S+) the latency of the lever press was also recorded (S+ response latency). If the initial response was incorrect (S- response) the number of incorrect lever responses made before switching the correct lever was recorded. Following a correct lever response, the water dipper was raised. The system measured whether the rat positioned itself above the dipper to obtain the water reward which provided a measure of the number of reinforcements obtained. In addition, the latency in obtaining the reinforcement in each trial was also recorded (SR+ latency). During the ITI periods, lever responses were measured to determine the number of ITI periods in which one or more lever presses occurred and the number of repetitive ITI lever response.

Table 4.10 Summary of dependent variables of the two-choice visual discrimination task

General measures of responding	
Total trials responded to	the total number of trial completed during each session. maximum = 100.
% Reinforcement obtained	the number of reinforcements obtained divided by the number of reinforcement delivered · 100: a measure of motivation.
Measures of stimulus control	
Discrimination ratio	the number of initial correct trial responses divided by the total trial responded to.
% ITI periods responded to	the number of ITI periods in which one or more lever responses was made divided by the total number of ITI periods · 100.
Measures of distribution	
Repetitive errors	the total number of incorrect trial responses which followed an initial incorrect response.
Repetitive ITI responses	the total number of ITI responses which followed an initial ITI response.
Measures of psychomotor speed	
S+ response latency	the latency in ms to make a correct initial trial response. Parameters examined include the overall S+ response latency (mean) for each rat, the variability (S.D.) for each rat, and the distribution of responses at different time intervals, i.e., S+ response occurring > 1s (very short), > 2s (short) and > 6s (pauses) after trial onset.
SR+ response Latency	the mean (\pm S.D.) latency in ms to obtain reinforcement for each rat.

- Results

For FOB and motor activity assessment, very few effects were observed:

- slight ataxia-like movements were observed in 1 out of 8 animals at the end of the first 8-hour exposure period in the 8,000 ppm group. In this group, 1 out of 8 animals also showed slight tremor after a single 8-hour exposure period.
- as regards the sensimotor domain, in the 8,000 ppm group, significant effects of treatment on the response to a touch on the flank were observed after the first 8-hour exposure period (not significant in a multi-comparison). A significant effect was also observed for the approach response after the third exposure period. Multiple comparisons showed that the mean response in the highest exposure group was significantly higher than the mean control score.
- in the 8,000 ppm group, there was a decrease (significant at the end of the first 8-hour exposure, not at the third) in body temperature.
- no effect was noticed in the motor activity assessment at all dose level tested.

For experiment II:

- A significant treatment-by-time interaction was observed for the number of repetitive errors in the 2,000 ppm group (due to a large extent to some animals displaying relatively high numbers of repetitive errors after the second 8-hour exposure period).
- Some effects on the psychomotor speed measurements were observed. The mean number of short (> 2s) response latencies differed between groups during the exposure period. Significant decreases in the mean number of short S+ response latencies in the 2,000 ppm group and a marginally significant decrease in the 8,000 ppm group were observed. Repeated ANOVA measurements were performed on these changes, expressed as the difference between the observed numbers on the day of the pre-test; a significant effect was

demonstrated only for the 8,000 ppm group. Significant increases in long S+ response latencies in the 8,000 ppm and in the 2,000 ppm groups were also observed, these differences being statistically significant in ANOVA for the 8,000 ppm group only.

Experiment III: High concentrations of cyclohexane were observed in the lipophilic brain compartment. Concentrations during uptake of cyclohexane in the brain were approximately ten times the blood concentration. The exposure levels used in this study were probably sufficient to saturate cyclohexane metabolism. It can also be concluded that there was no accumulation, elimination being fast and almost complete between each exposure (3·8 hours). These results are consistent with other kinetic studies. A summary of blood and brain cyclohexane concentration is given in **Table 4.11**:

Table 4.11 Blood and brain cyclohexane concentration following inhalation administration of 400–2,000 and 8,000 ppm in rats

Sampling time	Exposure level (ppm)	Cyclohexane concentration in blood (ng/ml)	Cyclohexane concentration in brain (ng/g)
Samples taken immediately after exposure			
2 hr	400	593	7,067
	2,000	3,433	34,000
	8,000	12,533	126,667
4 hr	400	540	6,250
	2,000	3,767	37,333
	8,000	14,333	153,333
1·8 hr	400	462	5,638
	2,000	3,300	36,250
	8,000	13,300	125,000
3·8 hr	400	452	5,600
	2,000	2,550	29,500
	8,000	14,875	143,750
Samples taken at different time points after a single 8-hour exposure			
0.5 hr	400	247	2,217
	2,000	1,550	15,667
	8,000	5,100	39,000
1 hr	400	141	942
	2,000	1,040	8,683
	8,000	4,633	32,333
2 hr	400	74	462
	2,000	537	4,067
	8,000	3,067	22,833
4 hr	400	43	278
	2,000	320	2,050
	8,000	1,630	12,000
8 hr	400	< 30	< 150
	2,000	90	583
	8,000	697	3,933

The blood and brain cyclohexane concentration measurements collected in this study will be used together with other information to develop and validate a PkPb model for cyclohexane.

Overall, for this study (experiments I and II), a NOAEL of 400 ppm can be assumed based on the increases observed in the response latencies observed in the discrete-trial two-choice discrimination task. This point of view is very conservative given that ANOVA analyses were only statistically significant at the dose of 8,000 ppm, but since the effects evaluated were very sensitive and highly variable, the effects observed in the 2,000 ppm dose can be considered as the beginning of the behavioural toxic effects. For this study, the authors concluded that "exposure at 400 ppm or 2,000 ppm of cyclohexane on a group basis did not induce neurobehavioral effects" but at 2,000 ppm, there were slight, statistically non-significant modifications of the response latencies and individual variations at 2,000 ppm. For this reason, 400 ppm (1,400 mg/m³) is considered to be a very conservative NOAEL.

Studies in humans

A study performed by TNO in 1998 (TNO, 1998c) was conducted in order to establish the neurobehavioral effects in human volunteers exposed to 250 ppm (860 mg/m³) of cyclohexane for 4 hours or to placebo: 25 ppm (86 mg/m³) only. Twelve human male volunteers (ages ranging from 20 to 39 years old) were exposed to the experimental conditions using a double blind, two-way cross-over design. The two tests were spaced 7 days apart. Subjects were tested using automated neurobehavioral tests and questionnaires prior to exposure, 45 and 165 minutes after the beginning and about 60 minutes after the exposure.

Venous blood samples were taken 10 minutes before the end of the exposure session to determine the internal level of cyclohexane exposure.

Cognitive functioning was assessed using selected tests from the Neurobehavioral Evaluation System including:

- attention: simple reaction time test, color word vigilance test, switching attention test,
- learning and memory: digit memory span test, spatial memory span test, pattern memory test, verbal memory test,
- psychomotor skills: hand-eye coordination test, finger tapping test,
- perceptual coding: symbol digit substitution test, pattern comparison test.

In addition, a computerised administered questionnaire designed to assess changes in mood and affect was also included.

This test was carried out in compliance with EC principles of GCP.

- Results

The mean blood concentration of cyclohexane was 55 ng/ml after 25 ppm exposure and 618 ng/ml for 250 ppm exposure.

The only effects observed were a significant improvement in performance under both treatment conditions the Hand-Eye Coordination Test: sinus condition; the Colour Word Vigilance Test and the Digit Memory Span Test during the first test day. The relevance of these parameters for the risk assessment is questionable, and these effects are not taken into account for the determination of a NOAEL. The only findings that could be treatment-related are "subjective" parameters like headaches and complaints of irritation of the eyes and throat which were more

frequently observed during and after exposure to 250 ppm of cyclohexane (compared with placebo).

For this study, a NOAEL of 250 ppm (860 mg/m³) can be assumed for human neurotoxicity, it should be noted that at this dose-level, subjective effects (headaches and signs of throat and eyes irritation) were observed more often than in the 25 ppm dose group.

Based on the TNO studies (1998a;b;c), Hissink et al. (1999) concluded that with the validated human PBPK model it was possible to calculate an exposure level which would lead to neurobehavioral effects in human. This dose would be 3,900 ppm leading to a NOAEL of 1,200 ppm. Because these values are derived from a model (not real tested values), it seems difficult to take them into account for the risk characterisation instead of the animal data.

4.1.2.2.4 Other information

According to Annex VI of the directive 67/548/EEC, cyclohexane fulfils the criteria for the classification Xn, R65: Harmful, may cause lung damage if swallowed. Cyclohexane has a kinematic viscosity of $1.259 \cdot 10^{-6}$ m²/s and a surface tension of 25.3 mN/m at 20°C.

4.1.2.2.5 Summary of acute toxicity (excluding acute neurotoxicity)

Most of the LD50 studies are old and were performed without GLP information, but the protocols and the results are correctly described in the reports or articles, so they can be considered reliable. All these studies suggest that cyclohexane is of low acute toxicity by the oral, inhalation or dermal routes. The low viscosity of cyclohexane justifies the classification Xn, R65.

4.1.2.2.6 Summary of acute neurotoxicity

The motor activity and behavioural assessment performed on rats revealed acute behavioural effects at high dose levels (8,000 ppm) but few at 2,000 ppm. According to the TNO studies, a NOAEL (very conservative due to the sensitivity of the method) of 400 ppm (1,400 mg/m³) can be set for neurotoxicity.

Acute neurotoxicity by inhalation was assessed in human volunteers, and no effects were observed at the highest dose tested: 20 ppm for 4-hour exposure period (860 mg/m³).

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin irritation

Two skin irritation studies using rabbits have been reported (Phillips Petroleum Company, 1982d; Jacobs and Martens, 1987), both performed in accordance with EEC Directive 83/467/EEC. The first, under a semi-occlusive dressing, gave a primary irritation score of zero 24 h and 72 h. The other, in a chamber and under a semi-occlusive dressing gave a mean erythema score, calculated over the 24 h and 72 h post-application period, below the threshold

value for classification as a skin irritant (1.93). A review of this study did however note that the erythematous reaction reached maximum severity at 5 days post-application (mean score 2.56). During this time, there was a gradual increase in dermal reaction for a further 144 h observation time (2.83). Overall, the irritation reactions were important and still present at the end of the study. Moreover, since cyclohexane is a solvent, defatting properties can also be expected. For this reason, a classification Xi, R38 was applied.

In addition, a repeated dermal application study in rabbits has been reported (Treon et al., 1943a). Repeated daily applications of undiluted cyclohexane onto an uncovered skin site for 14 days produced an initial erythematous response, gradually progressing to skin hardening, fissuring and bleeding with continued application. The authors reported healing of the lesions within one week once application of cyclohexane had ceased.

Eye irritation

Washed and unwashed primary eye irritation studies in rabbits are available (Phillips Petroleum Company, 1982e; 1982f). In the unwashed study, at one hour post instillation, corneal opacity, involving up to 25% of the cornea, was noted in one rabbit and iritis was noted in another rabbit. Conjunctival redness was noted in five rabbits with conjunctival chemosis in one rabbit. All ocular lesions had cleared within 24 hours and no conjunctival discharge was noted in any of the six animals.

Respiratory irritation

Acute inhalation exposure to cyclohexane at a nominal concentration of 32.88 mg/l of air did not appear to produce upper airway irritation in mice (Phillips Petroleum Company, 1982i). In this study, cyclohexane (vapour) was administered to 2 groups of 4 cd-1 male mice for 2 periods of 1 minute separated by 10 minutes. The only effect noted was a slight decrease (11.2% and 5.8%) in the respiratory rate of only one animal (out of four) in the two experiments. This animal also exhibited very slight respiratory pauses which may have been due to upper airway irritation. This study shows, as does the human study (TNO, 1998c) on volunteers where slight signs of irritation of the throat and the eyes were noted more frequently at 250 ppm (860 mg/m³) than at 25 ppm (86 mg/m³), that cyclohexane exhibits slight respiratory irritation properties but does not need classification for this end-point.

4.1.2.3.2 Studies in humans

Very little information has been reported on the irritation effects of cyclohexane in humans. The following information was cited in a UK HSE review of cyclohexane.

Skin irritation

Application of undiluted liquid cyclohexane to human skin for 1 hour produced erythema and weal formation.

Eye irritation

In a test involving self-reporting of eye irritation in human volunteers, most subjects reported no eye effects when exposed to 17.5 mg/m³ (5 ppm) cyclohexane vapour for 90 seconds. Some apparently reported very slight eye irritation, although the details are poorly reported (HSE,

1991). In the TNO study (1998c), human volunteers exposed to 250 ppm (875 mg/m³) of cyclohexane complained of irritation of the eyes and throat more frequently than those treated with 25 ppm (87.5 mg/m³).

4.1.2.3.3 Summary of irritation

From the available animal data, cyclohexane is considered to be a skin irritant. The regulatory assessment of tests gave results slight below the limits of classification but it was demonstrated that the irritant properties of cyclohexane were delayed and persisted until the end of observation period (16 days). Since defatting properties are also expected, this substance is classified Xn, R38. Cyclohexane is slightly irritant to the eyes both in animal and human experiments. Cyclohexane also exhibited slight respiratory irritant properties in mice and in humans, but as these effects were slight they were not considered important enough for classification purposes.

4.1.2.4 Corrosivity

From the studies presented in Section 4.1.2.3, it can be concluded that cyclohexane is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Skin sensitisation

Studies in animals

A modified Buehler test was performed by White Eagle Toxicology Laboratories (1996) to assess the sensitisation potential of cyclohexane (method B6 annexe V and according to EC and OECD GLP). Twenty guinea pigs (9 males and 11 females) were induced dermally with 10% cyclohexane (purity 99.98%) in ethanol and challenged with 10% cyclohexane in acetone. Concurrent negative controls (no cyclohexane) and positive controls (DNCB-0.1% in 50% ethanol at induction and 0.07% in acetone at challenge) were tested. It should be noted that a maximisation test was not required because of the very poor tolerance to intra dermal injection of solvents. During the induction phase, the response ranged from no redness (14/20 animals) to very faint redness on some tested animals (6/20 animals with a slight reaction). A very faint redness was observed 24 hours after the challenge application in 1/20 tested animals, no reactions were observed in other tested animals or negative controls. The incidence of sensitisation among cyclohexane induced and challenged animals was 0/20. The incidence of sensitisation among the DNCB induced and challenged animals was 8/10.

A higher challenge concentration could have been chosen (15% in acetone did not produce any dermal irritation) and there were only a few animals with dermal reactions during the induction phase, these findings reduce the significance of this test.

Studies in humans

There are no reports of skin sensitisation in humans attributed to this widely-used, high-tonnage material, so a very weak, if any, sensitisation potential can be anticipated for cyclohexane.

4.1.2.5.2 Respiratory sensitisation

No reports of allergic effects on the airways due to cyclohexane exposure have been found.

4.1.2.5.3 Summary of sensitisation

For skin sensitisation, the animal data available (one Buehler test performed according to EU and OECD guidelines) are sufficient to conclude that cyclohexane is not a strong skin sensitiser. Moreover, the lack of human case report for this high tonnage and widely used material strengthens this conclusion.

No data have been found concerning respiratory sensitisation and there are no indications that cyclohexane is a respiratory sensitiser.

Overall, according to the EU criteria, cyclohexane does not justify a classification for sensitisation properties.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Oral

No published data are available.

Inhalation

Rats

In a study specifically designed to investigate the ability of cyclohexane to produce an n-hexane-type neuropathy in rats (neuromuscular function was assessed: measurement of the hind limb spread on landing after being dropped from a 32 cm height), no outward manifestations of neuropathy, no effects on body weight gain, and no histopathological changes in nervous tissue were observed following inhalation of 1,500 ppm (5,250 mg/m³) or 2,500 ppm (8,750 mg/m³) cyclohexane as a vapour for 9-10 hours/day, 5-6 days/week for up to 30 weeks (Frontali et al., 1981).

The No Observed Adverse Effect Level (NOAEL) in rats was 2,500 ppm (8,750 mg/m³).

In a study performed by TNO in 1998, rats were exposed for 3 days to various concentrations of cyclohexane (see Section 4.1.2.2 for study design and experimental conditions).

The results of this study are summarised in the acute toxicity paragraph (Section 4.1.2.2).

No clinical signs of toxicity were observed in rats exposed by inhalation to 300-1,000 or 2,000 ppm (1,050-3,500 or 7,000 mg/m³) cyclohexane vapour for 6 hours/day, 5 days/week for 2 weeks. Only limited enzyme activity assessment performed on cerebral hemisphere homogenates revealed an apparent decrease in azoreductase activity in all cyclohexane-exposed groups, the significance of which is unclear (Savolainen and Pfäffli, 1980).

In a two-week inhalation range finding study (Haskell Laboratory, 1995a), CrI CD.BR rats (5/sex/group) were exposed (whole body in an exposure chamber) to 0-3,000-6,000 and 9,000 ppm (0-10,500-21,000 and 31,500 mg/m³) of cyclohexane (purity 99.97%). Nine exposures, each lasting six hours, were performed in total. The animals were weighed before treatment, clinical signs were checked before, during and after exposure, and common biochemical parameters and histological examinations were conducted at the end of the study. For neurotoxicity assessment, the animals were checked for alerting behaviour in response to a standardised auditory stimulus at least three times during each exposure. They were also submitted to an abbreviated Functional Observational Battery (FOB) before and after exposure on two separate days (test days 4 and 11). This assessment was also performed prior to the initiation of exposures to establish baseline measurements. During the FOB, the following parameters were assessed:

- in home cage: posture and palpebral closure,
- in open field: righting reflex, convulsions, gait characteristics, vocalisations, labored breathing, coordination, arousal and palpebral closure,
- during manipulations: approach and touch response, auditory response (clicker) and tail pinch.

This study was performed according to EPA guidelines and following EPA and OECD GLPs.

A slight but significant decrease in body weight gain was observed in males treated with 9,000 ppm. Except for a minimal increase in mitotic index figures detected in the hepatocytes of males at 6,000 ppm and higher and in females at 9,000 ppm, no other treatment related findings were observed for systemic toxicity. In particular, no modification in absolute and relative liver weights was noted in these studies. Based on these findings, a NO(A)EL of 3,000 ppm (10,320 mg/m³) can be assumed for systemic toxicity. For neurotoxic effects, diminished responses to stimulus were observed from day 2 at 9,000 ppm and from 7 exposures at 6,000 ppm. No effect was observed in FOB. A NOAEL of 3,000 ppm (10,320 mg/m³) can be assumed for neurotoxic effects in rats. This study served as a range-finding study for a 90-day inhalation toxicity study. It should be noted that this value is very conservative because the effects are very slight and may be of adaptive nature; this is taken into account in the risk characterisation.

In the 90-day inhalation toxicity study (Haskell laboratory, 1996a), cyclohexane (purity > 99.98 %) was administered to CD BR rats (20/sex/group for controls and high concentration and 10/sex/group for intermediary concentration groups) at doses of 0-500-2,000 and 7,000 ppm (0-1,750-7,000 and 24,500 mg/m³). Animals were exposed 6 hours/day, five days a week for 90 days (66 exposures) in exposure chambers (whole body exposure). Ten rats per sex were allowed a one-month recovery period for control group and 7,000 ppm groups. Periodically during the daily exposure, rats response to a standardised alerting stimulus were evaluated and recorded. Ophthalmoscopic examinations were conducted prior to initiation of exposure and near the end of the study. After 45 and 90 days of exposure, blood and urine were collected for evaluation of clinical pathology parameters. Gross pathology, organ weight, macroscopic and microscopic examinations were performed at the end of the study on organs commonly examined in this type of study.

This study was performed according to EPA guidelines and following EPA and OECD GLPs.

No treatment-related effects were observed on body weight, body weight gain, food consumption, urine analysis and clinical examinations. A slight decrease in SDH and LDH was observed in males and females at 7,000 and 2,000 ppm at both sampling times, these decreases not being considered toxicologically important. In males exposed to 7,000 ppm, a slight increase in adrenals weight was observed at the end of the recovery period. As this finding was not

observed at the end of the 90-day exposure, it was considered to be spurious. In the 7,000 ppm group, a statistically significant increase in the relative liver weight (4.001 vs 3.64 g in % of body weight) with hepatic hypertrophy was observed in males (10/10), concurrent with an increase in the incidence of centrilobular hypertrophy in both sexes (9/10 males and 5/10 females). This finding was partially reversible in the one-month recovery period (only 4/10 males with enlargement of the liver and no centrilobular hypertrophy observed), but in the interest of maintaining a conservative approach, this cannot be considered of no toxicological importance. For neurological effects, decreases in or absences of response to auditory stimulations were observed with a dose-response relationship from 500 ppm. In the 500 ppm group, there was a decrease in response on treatment days 61, 66, 67 and 68 (4/66). In the 2,000 ppm group, there was decrease in the response during 16 exposures and no response during 50 exposures. In the 7,000 ppm group, a decreased response was observed in one exposure and no response was observed in the other 65 exposures. These effects were transient, and as no clinical observations of compromised neurological function were detected they were considered to be due to a reversible sedation caused by cyclohexane.

For neurological effects, a NOAEL of 500 ppm (1,720 mg/m³) can be assumed regarding sedation during exposures at 2,000 ppm and higher (some effects observed at 500 ppm were very slight and occurred only in a few animals and only at the end of the study). For the hepatic effect, a NOAEL of 2,000 ppm (6,880 mg/m³) can be assumed regarding partially reversible effects observed in males at 7,000 ppm (24,080 mg/m³), as mentioned in the summary of the two-week study. These hepatic effects were slight and could be considered to be of an adaptive nature.

Additional groups of rats (12/sex/group) were treated in parallel with those of the previous study in order to assess neurotoxicity of cyclohexane in Functional Observational Battery (FOB), motor activity (MA) and neuropathology (NP) tests (Haskell 1996d). The experimental conditions were the same during the study except for the neurotoxicity specific assessment. Neurobehavioral evaluations were conducted prior to exposure and at week 4, 8 and 13. During each evaluation period FOB was performed prior to the MA test. Parameters assessed by FOB were:

- in home cage: posture, palpebral closure, writhing, circling, biting,
- in the removal from cage and handling evaluation: ease of removal, ease of handling, muscle tone, vocalisation, piloerection, bite marks on tail and/or paws, palpebral closure, fur appearance, lacrimation, salivation, exophthalmus,
- in the open field: righting reflex, labored breathing, convulsions/tremors, coordination, grooming, gait, locomotion, arousal, vocalisations, palpebral closure, defecation, urination,
- manipulations in the open field were: approach and touch response, auditory response, tail pinch response, forelimb grip strength, hind limb grip strength, foot splay,
- parameters assessed in the motor activity monitor were: defecation, urination, pupillary response.

For the motor activity assessment, the duration and number of movements were evaluated in six consecutive blocks of 10 minutes each for a 60-minute session.

At the end of the study, 6 animals/sex/group were selected for neuropathology, the controls and 7,000 ppm tissues selected were examined, and the intermediate doses tissues were saved and stocked. Sections of brain (forebrain, cerebrum, midbrain, pons, medulla and cerebellum), spinal cord (cervical and lumbar), sciatic nerve, gasserian ganglia, cervical and dorsal root fibers and ganglia (DRF&G), cervical and lumbar ventral root fibers (VRF) and gastrocnemius muscle were

sectioned, embedded and stained with different techniques in order to demonstrate any neurologic lesions.

Like the other groups (sub-chronic study and developmental study), a sedative effect was observed at doses of 2,000 ppm and higher characterised by a decrease in the mean response to an alerting stimulus (diminished to no response for the highest dose). This effect was transient since no effects were observed immediately after removal from the exposure chamber. No effects were observed during the FOB and MA assessment. Histologically, no treatment-related findings were observed, the only lesions observed being identical in character and severity to those observed in controls. These have already been described as occurring spontaneously in the rat. For this study, a NOAEL of 500 ppm (1,720 mg/m³) can be assumed, based on the transient sedative, effect observed at 2,000 ppm (6,880 mg/m³) and higher.

Mice

A comparative rats/mice 2-week study was performed by Haskell laboratory in 1995a (cf. rat study for experimental conditions) on Crl CD1 (ICR) BR mice.

A slight increase in lungs weight was observed in males treated with 9,000 ppm. An increase in the relative and absolute liver weight was observed at and above 6,000 ppm in both males and females. This was associated with slight centrolobular hypertrophy and increased mitotic figures in males at 9,000 ppm and in females at all test concentrations. No NOAEL can be set for systemic toxicity in mice. The LOAEL is 3,000 ppm (10,500 mg/m³), which is very conservative value since the effects might be considered to be of an adaptive nature. As in rats, diminished responses to stimulus were observed from day 2 at 9,000 ppm and from 7 exposures at 6,000 ppm, these symptoms being associated with behavioural changes such as jumping and slow circling. No effect was observed in FOB. The NOAEL for neurotoxicity in mice is 3,000 ppm (10,320 mg/m³) in this study. This study served as a range-finding study for the 90-day inhalation toxicity study in mice.

A 90-day inhalation toxicity study (Haskell Laboratory, 1996b) was performed following the two-weeks range finding study, and was comparable in experimental conditions to that performed on rats (cf. ref Haskell Laboratory, 1996a).

After a stimulus, the animals of the 500 ppm group reacted as controls. In the 2,000 ppm group, a decrease in or an absence of response was observed from the third exposure onwards, the effects appearing to get worst with time (more and more no-response with increasing numbers of exposures). In the 7,000 ppm group, there was an increase in the incidence of decreased response, absence of response and hyperactive state from test day 4 to test day 30. From test day 30 to the end of the study, the response to the stimulus was impossible to determine due to the hyperactive state of the animals. These symptoms were observed just after exposure but were reversible until the next exposure. The most frequently described symptoms were: abnormal gait or mobility, excessive grooming, hyperactivity, hyper reactivity, spasms, aggressivity, hypo-activity and ruffled fur.

In males, haematological abnormalities were observed from 500 ppm, these symptoms (increase in RBC - increase in Hb - increase in Ht and decrease in platelets) were not always statistically significant and not always dose-related. In females, increases in RBC, Hb and Ht were only observed at 7,000 ppm. Variation in the haematological parameters occurred for all animals at the 7,000 ppm dose level, but could not be explained in relation to the lack of systemic symptoms of dehydration. They were considered to be of no toxicological importance.

An increase in absolute and relative liver weight was observed in males at 7,000 ppm (absolute: 1.504 g vs 1.275 g). Only the relative weight was increased in females. No concomitant histological findings were observed. These results were not in accordance with those found during the two-week range finding test (histological findings from 3,000 onwards in females and at 9,000 in males) but did not interfere with the determination of a NOEL.

For neurologic effects, a NOAEL of 500 ppm (1,720 mg/m³) can be assumed regarding signs of sedation observed in animals at 2,000 ppm (6,880 mg/m³). For systemic toxicity, a NOAEL of 2,000 ppm (6,880 mg/m³) can be assumed based on the effects observed in the liver at 7,000 ppm.

Rabbits

Ten inhalation experiments on rabbits were reported in a 1943 paper. The concentration and time relationships ranged from 435 ppm (1.47 mg/l) for 1,040 hours to 26,572 ppm (89.6 mg/l) for 1 hour, cyclohexane was administered as a vapour. No clinical signs of toxicity were observed following exposures to 435, 786 or 3,330 ppm for 6 hours/day, 5 days/week for 10 weeks, or to 435 ppm for 8 hours/day, 5 days/week for 26 weeks. Exposure to 7,444-18,565 ppm (25-63 mg/l) for 6 hours/day, 5 days/week for 2-5 weeks produced some deaths and a concentration-dependent increase in the severity of a number of clinical symptoms - nose rubbing, conjunctival injection, weight loss, salivation, diarrhoea, lethargy, narcosis, loss of coordination, temporary paralysis of the legs, tremors, rapid laboured respiration and cyanosis. Weekly haematological assessments on all the animals revealed no effects due to cyclohexane exposure. No histopathological effects were seen in rabbits exposed to 435 ppm for 6 hours/day, 5 days/week for 10 weeks. At 786 ppm barely demonstrable microscopic changes were observed in the liver and kidneys. Higher concentrations resulted in generalised vascular endothelium injury and widespread tissue inflammation and degenerative changes. In this study no biochemistry was performed (Treon et al., 1943b).

Rabbit NOAEL = 435 ppm, 8 hours/day, 5 days/week for 26 weeks (1,500 mg/m³).

Rabbit LOAEL = 786 ppm, 6 hours/day, 5 days/week for 10 weeks (2,700 mg/m³).

Monkeys

Concurrently to the series of rabbit studies described above, 1 monkey was exposed to 1,243 ppm (4.19 mg/l) cyclohexane vapour for 6 hours/day, 5 days per week for 10 weeks (Treon et al., 1943b). With the exception of weight loss, no clinical signs of toxicity or histopathological effects were observed in this one animal.

Dermal

Rabbits

In a 1943 study, one rabbit received repeated daily applications of undiluted cyclohexane on uncovered skin over a period of 14 days, giving a total dose of 180.2 g/kg (Treon et al., 1943a). The result was irritation and thickening of the skin but no fatality. The authors reported that no narcosis or convulsions were noted at any time, but the rabbit did lose weight during the applications and for one week following treatment. Widespread vascular damage and tissue inflammation and degeneration was observed when histopathological examination of internal organs was performed after an unstated period of time.

It was not possible to determine a dermal NOAEL from this limited study.

Other routes

Rats

Bernard et al. (1989) evaluated the nephrotoxicity of cyclohexane in female rats. These animals were injected with 0.375, 0.75 and 1.5 g/kg of cyclohexane via intraperitoneal route five times per week for 2 weeks. They noted an increase in β 2-microglobulinuria which was dose- and time-dependent. The renal concentration ability was depressed only at the highest dose. The same nephrotoxicity was observed after injection of 0.4 g/kg cyclohexanol, so the authors suggested that cyclohexane nephrotoxicity was due to cyclohexanol.

4.1.2.6.2 Studies in humans

One paper from 1993, concluded that cyclohexane exposure at the concentrations studied (geometric mean 27 ppm (94.5 mg/m³), highest concentration observed 274 ppm (959 mg/m³)) induced no significant increase in the prevalence of subjective symptoms or in the haematological and serum biochemical parameters of the liver and kidney functions. The authors of this study pointed out that the exposure intensity was low, well below the 1993 occupational exposure limits (Yasugi et al., 1994).

The 1991 HSE review on cyclohexane raised the point that cyclohexane, associated with other chemicals, has been implicated by some authors in the peripheral neuropathy seen among Italian shoe factories, printing plants and paint workers, cyclohexane frequently being a major component of the solvents and adhesives used (De Rosa et al., 1985; Mutti et al., 1982; Franco et al., 1979). However, the HSE review pointed out that such workers received mixed exposure to various solvents, including n-hexane, and concluded that the experimental evidence suggests that the neuropathy was caused by the n-hexane component of the solvents and adhesives used in these industries (HSE, 1991).

A study was carried out by Yuasa et al. (1996) in a luggage factory. The purpose was to assess the neurotoxicity of cyclohexane during occupational exposure. 18 women (aged from 18 to 56) were exposed for eight hours a day to glue containing 75.6% cyclohexane, 12% toluene and 0.9% n-hexane. Twelve of them had been working with n-hexane for 0.3-20 years and had stopped this type of work 0.7-2.6 years before the first investigation. In order to prevent n-hexane exposure related effect, a further group of nine workers took part in a follow-up study, one year after the first study. A control group of 18 non-exposed women was chosen from among medical students and clerical workers. The evaluation of exposure was made by urinary measurement of cyclohexanol after eight hours of exposure and in accordance with the Yuasa method (1994). Depending on the past and present exposure data, the workers were divided into higher (n = 7) and lower (n = 11) exposure groups, and the nerve conduction velocities (NCV) of each group were compared. The limit between the high exposure group and the low exposure group was a cyclohexane concentration of 100 ppm.

Some neurologic indices were measured:

- MNCV: maximum nerve conduction velocity of the motor fibers of the ulnar nerve between the wrist and elbow and of the common peroneal nerve between the knee and ankle,
- MDL: motor distal latency of the ulnar nerve and common peroneal nerve,
- SNCVp: proximal conduction velocity of sensory fibers of the ulnar nerve between the elbow and wrist,
- SNCVd: distal conduction velocity of the sensory fibers of the ulnar nerve between the wrist and 5th metacarpophalangeal joint and of the sural nerve between the calf and foot,
- amplitude: amplitude of maximum sensory nerve action potential of the ulnar nerve and sural nerve.

These variables were measured on the dominant arm and the leg of the same side by a standard method (with electromyography). The nerves were supramaximally stimulated with a single pulse of 0.1 ms (for motor nerves) or 0.2 ms for sensory nerves at a frequency of 2 Hz. Individual external exposure ranged from 5 ppm to 211 ppm (17.2 to 725 mg/m³) with a mean of 28 ppm (96 mg/m³). Urinary cyclohexanol ranged from 0.12 mg/l to 8.23 mg/l with a mean of 0.55 mg/l. Very slight non-significant differences were noted in the frequency of the subjective symptoms: for fatigue, the incidence was 9/18 vs 4/15 in the exposed and the non-exposed, respectively. Headaches were observed in 10/18 of the exposed workers versus 7/15 of the non-exposed. Feelings of dizziness and uneasiness were noted in 7/18 and 8/18 of the exposed people and in 4/15 and in 5/15 of those unexposed. For neurological indices, no differences were noted in MNCVs and SNCVs. There were slight differences in the ulnar and peroneal MDLs (shorter in the workers than in the controls) in the first study. No neurophysical indices were affected in the second study. It can be assumed that the slight effects observed during the first study were certainly attributable to the precedent exposure to n-hexane. Overall, there was no evidence of neurotoxicity during a relatively short period of exposure at low concentrations (below the time weighted average threshold limit values of the US (150 ppm)).

4.1.2.6.3 Summary of repeated dose toxicity

Recent studies performed on cyclohexane via the inhalation route in mice and rats showed that slight liver effects were induced after sub-acute or sub-chronic exposure. Increases in mitotic index figures and in absolute and relative liver weight and centrolobular hypertrophy were noted in both rats and mice at dose levels between 6,000 and 7,000 ppm (in the 28-day and the 90-day studies, respectively). The NOAEL for hepatic effect is estimated to be 2,000 ppm (6,880 mg/m³). This value is very conservative since the effects observed in the liver from 6,000 ppm upwards may be of an adaptive nature.

For neurotoxicity, the same studies assessed narcotic properties and motor activity changes. In mice and rats, reversible changes of responses to stimulus (decreases or no reactions) were observed at doses of 6,000 ppm in the 28-day studies and at doses of 2,000 ppm in the 90-day studies. This effect can be considered as an acute effect, and the NOAEL was estimated to be 500 ppm (1,720 mg/m³). This value will be taken into account in the risk characterisation of acute neurotoxicity.

In older studies in rabbits, liver and renal effects were observed at dose levels of about 800 ppm, leading to a NOAEL of about 500 ppm. Due to the limitations of such studies these effects are not taken into account in the risk characterisation, but are of the same order of magnitude as the results observed in the most recent studies on rats and mice.

In recent studies in humans, no effects were seen at concentrations ranging from 27 to 274 ppm.

Although the potential of cyclohexane to produce peripheral neuropathy in exposed workers has been questioned, all the data come from mixed exposures where n-hexane, a well known neurotoxic agent, was also present. As the metabolic pathway of cyclohexane is clearly different from that of n-hexane (particularly without production of 2,5-hexadione), it can be stated that cyclohexane is not in itself neurotoxic for the peripheral nervous system.

As some solvents are supposed to cause chronic encephalopathy (also called "organic brain syndrome") concerns have been raised about cyclohexane. This effect has not been studied experimentally or epidemiologically. However, there are no published indications that cyclohexane can cause this effect.

According to EU criteria for classification and labelling, cyclohexane is classified R67 due to its narcotic effects.

Table 4.12 Summary of repeated dose toxicity studies

Systemic (with or without neurotoxicity assessment)			
Species	Administration protocol	Objectives of the tests	NOAEL
Rat	inh. 30 weeks, 9-10 h/d, 5-6 d/w	ability of cyclohexane to produce neuropathy	2,500 ppm (8,600 mg/m ³)
Rat	inh. 2 weeks, 6 h/d, 5 d/w	effect of cyclohexane on cerebral enzyme activity	2,000 ppm (6,880 mg/m ³)
Rat	inh. 2 weeks, 6 h/d, total 9 exposures	neurotoxicity assessment, behaviour, functional observational battery (FOB).	3,000 ppm (10,320 mg/m ³)
		systemic toxicity (biochemical and histological toxicity).	3,000 ppm (10,320 mg/m ³)
Rat	inh. 90 d, 6 h/d, 5 d/w	systemic toxicity : clinical, haematology, biochemical and histology	2,000 ppm (6,880 mg/m ³)
		neurobehavioral effects	500 ppm (1,720 mg/m ³) (acute effect)
Rat	inh. 90 d, 6 h/d, 5 d/w	FOB, motor activity and neuropathology	500 ppm (1,720 mg/m ³) (acute effect)
Mouse	inh. 2 weeks, 6 h/d, total 9 exposures	neurotoxicity assessment, behaviour, functional observational battery (FOB)	3,000 ppm (10,320 mg/m ³)
		systemic toxicity (biochemical and histological toxicity)	< 3,000 ppm (< 10,320 mg/m ³)
Mouse	inh. 90 d, 6 h/d, 5 d/w	systemic toxicity: clinical, haematology, biochemical and histology	2,000 ppm (6,880 mg/m ³)
		neurobehavioral effects	500 ppm (1,720 mg/m ³) (acute effect)
Rabbit	26 weeks, 8 h/d, 5 d/w	systemic toxicity (no biochemical investigation)	435 ppm (1,500 mg/ m ³)
Neurotoxicity			
Species	administration protocol	objectives of the tests	NOAEL
Rat	1) inh. 3·8 h/exposure	neuromuscular, sensor motor, convulsive, excitability and activity	2,000 ppm (6,880 mg/m ³)
	2) inh. 3·8 h/exposure	discriminative task	400 ppm (≈1,400 mg/m ³)
Rat	inh. 6 h	schedule-controlled operant behaviour	2,000 ppm (6,880 mg/m ³)

4.1.2.7 Mutagenicity

4.1.2.7.1 *In vitro* studies

In vitro assays

- Cyclohexane (> 99% pure) was tested in a pre-incubation modification of a standard Ames test (Mortlemans et al., 1986). *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) were incubated with and without metabolic activation in DMSO. Two metabolic activation systems were used, one with SD rats livers and the other with Syrian hamster livers. Doses of cyclohexane ranged from 0 to 10,000 µg/plate.

Results: Signs of toxicity were noted from 3,333 µg/plate for TA1537 and TA98 and at 10,000 µg/plate for TA100 and TA1535. There was no evidence of reverse mutation for any dose tested with and without metabolic activation.

- Cyclohexane did not exhibit mutagenic properties in a standard Ames test reported by Mc Cann et al. in 1975. In this publication, only a table of results was available with no information about the toxicity, the method used and the range of concentrations.

- In a Mouse Lymphoma Assay, cyclohexane, solubilised in desionised water, was tested at doses ranging from 313 nl/ml to 10,000 nl/ml (250 µg/ml to 7,800 µg/ml) (API, 1986). The method used was equivalent to the guidelines.

Results: cytotoxicity was seen at 10,000 nl/ml (7,800 µg/ml) with and without metabolic activation. Without metabolic activation the percentage of relative growths ranged from 39 to 59 % without dose-effect relationship. There was no evidence of forward mutation at any dose. With metabolic activation, the percentage of relative growths ranged from 46 to 64% and was not dose related. There were very slight increases in mutant frequency in four treatments, but not dose related, and it was decided to confirm this result with another test. Doses ranging from 3,000 to 8,000 nl/ml (2,340 to 6,240 µg/ml) were tested in the second trial (cytotoxicity was found at 9,000 nl/ml (7,020 µg/ml)). The percentage of relative growths ranged from 23 to 69%. In this trial the results were clearly negative. Overall, this test can be considered as negative with and without metabolic activation.

- In another Mouse Lymphoma Assay, cyclohexane (100% pure) in DMSO was tested at doses ranging from 8 µg/ml to 100 µg/ml (Phillips, 1982g). The method used was equivalent to guidelines studies.

Results: only a table of results is available for this test. Without metabolic activation, no toxicity was seen at any dose tested, the percentage of total survival was between 90 and 140%, and no increases in forward mutation frequencies were observed. With metabolic activation, the percentage of growth inhibition rose to 65% for the highest dose tested (100 µg/ml) and no effects were seen on mutation frequency. This well-performed test is considered negative in the limits of the range tested.

- In an *in vitro* Sister Chromatid Exchange test, CHO cells were incubated with various doses of cyclohexane (0.25 µg/ml to 25 µg/ml in DMSO) with and without metabolic activation (Phillips, 1982h). This study was performed according to a method in keeping with the guidelines, the higher dose tested corresponding to a complete growth inhibition of the cell culture (25 µg/ml). No effect was seen within the range of the doses tested and the test is considered negative.

- Cyclohexane (99.8% pure) was tested at doses of 10^{-2} - 10^{-3} and 10^{-4} M in DMSO in an Unscheduled DNA Synthesis test (Perocco, 1982). Human lymphocytes were cultured for four hours in the presence or absence (controls) of cyclohexane. The effects on the DNA synthesis were measured through cellular [3 H]TdR uptake. Short-term toxicity (4 h culture) was determined by the trypan-blue staining technique. Cultures were grown in the absence or in the presence of a rat liver metabolising system (S9 mix).

Results: cyclohexane caused a marked inhibition of [3 H]TdR uptake in the S-9 mix-lacking cultures while the corresponding cellular viabilities were unaffected. No effect was seen with metabolic activation. According to the authors, these data suggest that cyclohexane exercises a cytotoxic action which is not immediately followed by cellular death, presumably because they inhibit some step of the DNA synthesis and/or interfere with the thymidine uptake processes.

However, the effects seen without metabolic activation were not dose-effect, solvent controls and negative controls were of a great variability, decrease of the uptake for the highest dose was within the values of the controls. In the method, no definition of positivity or negativity was given. All these inconsistencies reduce the reliability of this study. No conclusion can be drawn.

- Cyclohexane was tested in a DNA cell binding assay (DCB - Kubinsky et al., 1981) at doses of 10 and 100 μ M. Cyclohexane was tested alone, mixed with lysozyme, mixed with liver extract and mixed with lysozyme and liver extract. Positive control was MMS and negative control was the culture middle only. The results are expressed as a "binding percentage". If this percentage is $> 1\%$, the substance was considered positive.

Results: cyclohexane was found negative when tested alone in the groups treated with liver extract and –with lysozyme and liver extract. A positive finding (1.6% only) was found in the group treated with cyclohexane + lysozyme at the highest dose (100 μ M). This result is considered doubtful because this is a very slight increase and also because this effects is not found in the group - cyclohexane + lysozyme + liver extract.

Table 4.13 *In vitro* genotoxicity data on cyclohexane

Assay	Strains/type	Metabolic activation	Result	Comment	Reference
<i>Salmonella typhimurium</i>	TA1535, TA1537, TA97, TA98, TA100	with and without Aroclor induced rat and hamster liver S-9	negative	a pre-incubation assay was also negative	Mortlemans et al. (1986)
<i>Salmonella typhimurium</i>	TA1537, TA1535, TA98, TA100	with and without Aroclor induced rat liver S-9	negative	brief report	McCann et al. (1975)
Mouse lymphoma assay	L5178Y (TK locus)	with and without Aroclor induced rat liver S-9	negative	comprehensive report	API (1986)
Mouse lymphoma assay	L5178Y (TK locus)	with and without Aroclor induced rat liver S-9	negative	summary report	Phillips (1982g)
Sister chromatid exchange assay	CHO	none	negative	full report not seen	Phillips (1982h)
Unscheduled DNA synthesis	human lymphocytes	with and without phenobarbitone induced rat liver S-9	negative	lymphocytes exposed to 0.1-10 mM cyclohexane for 4 h	Perocco (1983)
DNA binding to E Coli		E Coli Q3 cells none	doubtful	non standard test protocol; negligible activity detected for DNA pre-treated with 10 or 100 μ M cyclohexane except in the group treated	Kubinski et al. (1981)

4.1.2.7.2 *In vivo* studies

In vivo cyclohexane has been studied in a rodent bone marrow cytogenetic assay (American Petroleum Institute, 1982). Groups of 10 male and female Sprague Dawley rats were exposed by inhalation to atmospheres of 0, 97, 307 and 1,042 ppm for 6 hours per day for 5 days (350-1,050–3,650 mg/m³). Samples of bone marrow cells were taken for cytogenetic analysis 6 hours after completion of the final dose. A positive control, triethyleneamine, showed a significant increase in structural aberration frequency. For cyclohexane a small but statistically significant increase in numerical aberrations was recorded in low and medium dose females, and pooled data at the low dose groups of both sexes. There was no information on general toxicity; no decrease on mitotic index was seen at all the doses tested. However, the authors of the report concluded that the lack of a dose-related response indicated that these increases were not of biological importance. Moreover, the numerical aberrations parameter had often shown great variation in this laboratory, having no statistical significance even for positive controls (numerical data is not available). It can be considered that cyclohexane does not produce chromosomal aberration in the conditions of this test.

A *Drosophila* sex linked recessive lethal assay gave negative results (Shetty and Ragaswamy, 1984).

4.1.2.7.3 Summary of mutagenicity

The available *in vitro* mutagenicity studies do not indicate that cyclohexane has genotoxic properties. For *in vivo* studies, negative results were found for a *drosophila* sex linked recessive lethal assay. Slight effects unrelated to dose were noted in a micronucleus test, but these were considered to be of no biological importance.

4.1.2.8 Carcinogenicity

There is only one study where cyclohexane was assessed for its tumorigenic potential in a multistage initiation-promotion test on mouse skin (Gupta et al., 1990)

In the first part of this study, single or repeated (24-hourly for 3 weeks) application of 100 µl of cyclohexane (99.5% purity) resulted in stimulation of ornithine decarboxylase activity, a marker of cell proliferation.

In the second part, groups of 20 mice were initiated with DMBA and seven days later promoted with repeated skin application of TPA plus cyclohexane (100 µl (78 µg)) (a) or cyclohexane alone (b). Local tumours were seen in 45% of mice in (a) and 10% in (b) (see **Table 4.14**).

Table 4.14 Tumour promoting potential of cyclohexane according to the initiation-promotion protocol

Group	Initiation	Promotion	Cumulative no. of tumour	% Tumour bearing animals
1	DMBA	TPA+ Cyclohexane	21	45
2	DMBA	cyclohexane	2	10
3	DMBA	TPA	80	100
4	acetone	TPA	∅	∅
5	none	none	∅	∅
6	DMBA	TPA	∅	∅
7	DMBA	acetone	∅	∅

Mice in groups 1, 2 and 3 were topically initiated with 0.2 μmol (51.2 μg) of DMBA dissolved in 0.2 ml acetone. Seven days after initiation, group 1 mice were promoted three times a week with 5 μg TPA in 0.2 ml acetone for 2 weeks followed by thrice a week applications with 100 μl cyclohexane; mice in group 2 were promoted thrice a week with 100 μl cyclohexane alone; group 3 mice were promoted thrice a week with 5 μg TPA in 0.2 ml acetone; in group 7, mice were promoted with 0.2 μl acetone thrice a week. In group 4, acetone was used for initiation and TPA for promotion as usual. A set of animals was kept as untreated controls, i.e. group 5. In group 6, the animals were initiated by DMBA and promoted by TPA, like group 1 for 2 weeks only. There were 20 mice in each treatment group. The experiment was run for 45 weeks. Mortality was very low, 1 to 3 animals dying in each group.

In the same experiment, local tumours were seen in all positive control animals treated with DMBA then TPA. The results of group 3 are curious when compared with those obtained with group 1 (same treatment protocol with or without cyclohexane). It is also regrettable that no group treated with cyclohexane only (without promotion) was considered. Unfortunately, the ornithine decarboxylase activity was checked for cyclohexane treated animals only; it would have been interesting to check the activity of this enzyme for all the groups treated.

In this study, according to the authors, cyclohexane is effective as a weak tumour promoter, particularly a stage II tumour promoter. Given the uncertainties of the results and of the method, the significance of this study is questionable.

Cyclohexane was found to be negative in a cell transformation test performed on a SA7/SHE system at doses ranging from 62 $\mu\text{g}/\text{ml}$ to 1,000 $\mu\text{g}/\text{ml}$ (Heidelberger et al., 1983). No transformation activity was found at any dose tested.

Summary of carcinogenicity

No data support the view that cyclohexane is a genotoxic substance. It was demonstrated in a questionable study that cyclohexane might have a weak promotion potential. Despite the lack of a conventional two-year carcinogenic test, we can consider that cyclohexane is unlikely to be carcinogenic.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Two-generation reproduction studies (rat)

A two-generation study was performed on rats (Haskell Laboratory, 1997e) using the experimental procedure described in the 90-day inhalation toxicity test. Groups of 30 animals/sex/dose were exposed to 0-500-2,000 and 7,000 ppm (0–1,720–6,880–24,080 mg/m³) cyclohexane in exposure chambers, 6 hours a day for 5 days a week, 10 weeks before mating, during gestation and lactation. The age of the rats at the start of exposure was about 56 days for the P1 generation and 26-28 days for the F1 generation. Exposure duration was 10 weeks before mating until sacrifice of the P1 generation and 11 weeks before mating until sacrifice of the F1 generation. Gravid females were not exposed from D21 of gestation until D4 of lactation. From D5 of lactation until weaning the neonates could only be exposed by maternal milk. At Post Partum Day (PPD) 25, thirty F1 animals/sex/dose were chosen to produce the next generation, treatment was continued 11 weeks before mating and during gestation. Fertility parameters were calculated. Twenty F1 and F2 weanlings/sex/concentration were selected for gross post-mortem examination. After litter production, all parents were given a gross pathologic examination, testes-epididymides-prostate-seminal vesicles-coagulating gland and pituitary were collected for males whereas ovaries-uterus-cervix-vagina and pituitary were collected for females. Tissues from 7,000 ppm groups were examined microscopically and, in addition, gross lesions and target organs from adult rats in all dose groups were microscopically examined. This study was performed according to EPA and OECD GLP.

From 500 ppm, there was an increased incidence of diminished response to a stimulus during exposure, this finding being significant at 2,000 ppm and higher (at 500 ppm there was only diminished response in seven observations in 235 days of treatment). At 7,000 ppm, major effects were observed on body weight, body weight gain and food efficiency:

- decrease in mean body weight of F1 male rats,
- decrease in mean body weight gain of F1 male rats on the whole period 1-120 test days,
- decrease in body weight of P1 and F1 females during pre-mating (at the end of the period),
- decrease in mean body weight gain of P1 and F1 females during pre-mating (on the whole period 1-71 test days), during gestation,
- statistically significant reduction in the mean body weight of P1 females in the 7,000 ppm group throughout gestation. However, there were no statistically significant differences in overall mean body weight gain for this group during gestation, suggesting that the reduction in mean gestation body weight was probably due to pre-existing body weight deficits established during the pre-mating period (see **Table 4.15**). The same findings were evidenced in the F1 generation (see **Table 4.16**),
- decrease in mean food efficiency of P1 and F1 females during lactation,
- decrease in food consumption of P1 females during lactation,
- decrease in mean body weight of F1 females during lactation.

The only effects on reproduction functions were limited to a decrease in mean pup weight for both the F1 and F2 generations at dose level of 7,000 ppm. This was statistically significant between Post Partum Days (PPD) 7-25. During these days, the pups were fed only maternal milk (treated with cyclohexane from PPD5 to weaning); this effect could therefore be due to

cyclohexane via lactation. There was also a slight increase in incidence of pro-static inflammation at 7,000 ppm in P1 and F1 adults, but this was considered as incidental due the lack of severity and the reported common occurrence in this species. There was also a slight but significant decrease in the mean percentage of born alive animals in the F1 litters dosed with 7,000 ppm, but given that the value was still in the range of historical controls and that this effect was not dose-related, this was considered as incidental (see **Table 4.17**).

Table 4.15 Mean body weights and mean body weight gains (g) of P1 female rats during gestation

Concentration	0	500	2,000	7,000
Gestation days	Mean body weight			
0	288.4 (27.4)	288.2 (24.3)	286.3 (23)	268.6 (24.2) *
7	308.7 (29.9)	310.8 (22.6)	307 (23)	285.2 (24.5) *
14	333.6 (31.7)	334.6 (23.4)	329.6 (24.7)	307.9 (27.8) *
21	418.4 (42.7)	421.6 (25.9)	415.8 (28.8)	391.1 (31.2) *
Gestation days	Mean body weight gain			
0-7	20.3 (5.9)	22.6 (6.6)	20.7 (7.4)	16.6 (6.9)
7-14	24.9 (5.9)	23.9 (5.2)	22.6 (5)	22.7 (6.9)
14-21	84.9 (18.9)	86.9 (12.3)	86.2 (17.1)	83.1 (10.2)
0-21	130.0 (23.9)	133.4 (14.3)	129.5 (21.6)	122.5 (13.9)

Standard deviation is reported in parentheses

* Statistically significant difference from control ($p \leq 0.05$) by one-way analysis of variance and Dunnett's test

Table 4.16 Mean body weights and mean body weight gains (g) of F1 female rats during gestation

Concentration	0	500	2,000	7,000
Gestation days	Mean body weight			
0	301.5 (28.4)	300.5 (30.3)	310 (33.3)	277.7 (27.6) *
7	328.3 (31.9)	325.7 (31.7)	333.7 (35)	299.3 (26.5) *
14	355.2 (33.8)	354.5 (32.8)	363.3 (36.4)	328 (25.2) *
21	445.2 (34.7)	439.6 (35.7)	460.5 (42.1)	415.6 (32.8) *
Gestation days	Mean body weight gain			
0-7	26.8 (5.3)	25.3 (5.8)	23.8 (6.9)	21.6 (5.2) *
7-14	26.8 (9.5)	28.8 (6.2)	31.3 (4.6)	28.7 (4.7)
14-21	90.1 (15.2)	85 (21.7)	96.7 (16.2)	87.6 (16.2)
0-21	143.7 (19.8)	139.1 (24.4)	150.5 (21)	137.9 (17.3)

Standard deviation is reported in parentheses

* Statistically significant difference from control ($p \leq 0.05$) by one-way analysis of variance and Dunnett's test

Table 4.17 Mean pup numbers and survival : F1 generation

Concentration (ppm)	0	500	2,000	7,000
Survival (%)				
Sex ratio (males)	0.5	0.55	0.46	0.5
Gestation index ^{a)}	100	96.3	100	96.4
Mean % born alive	100	96.3	99	98.1 *
0-4 Day viability	97.9	99.2	99.3	99.3
Lactation index ^{b)}	100	99.5	100	99.5
Litter survival ^{c)}	100	100	100	100

a) Percentage litters delivered having at least one live pup

b) Mean percent survival from day 4 post culling to day 25

c) Percentage litters born with at least one pup alive on day 25

* Statistically significant difference from control ($p \leq 0.05$) by Jonckheere's test

No statistically significant differences from control in gestation index or litter survival ($p \leq 0.05$) by Cochran-Armitage test

For this study, a NOAEL of 500 ppm (1,720 mg/m³) can be assumed for systemic toxicity (based on sedative effects observed at 2,000 ppm and higher). For reproductive toxicity, a NOAEL of 2,000 ppm (6,880 mg/m³) can be derived from the decreases in pup body weight observed at 7,000 ppm (in the presence of parental toxicity).

4.1.2.9.2 Developmental toxicity studies

Rats

A pilot inhalation developmental study of cyclohexane in rats was performed by Haskell (1997c). Four groups of eight pregnant CrI:CD BR rats were exposed whole-body to concentrations of 0-3,000-6,000 and 9,000 ppm (0-10,320-20,640-30,960 mg/m³) cyclohexane from Gestational Day 7 to 16 (D1 was the copulation day). Dams were sacrificed on GD22 and examined for gross pathologies; implantations and resorptions were counted and their relative positions recorded; fetuses were weighed and examined externally for alterations. This study was performed according to EPA and OECD GLP.

Maternal effects were limited to a reduction in overall maternal bodyweight gain, overall food consumption and diminished response of animals to a sound stimulus during exposure to 6,000 ppm and higher. No effects were observed in the pups. The NOAEL for this study is assumed to be 3,000 ppm (10,320 mg/m³) for the dams and 9,000 ppm (30,960 mg/m³) for the pups.

This study served as a range-finding study in order to perform a more complete study (carried out during the 90-day inhalation toxicity test with the same exposure schedule). Four groups of 25 CrI:CD BR rats were exposed whole body to atmospheric concentration of 0-500-2,000 and 7,000 ppm cyclohexane (0-1,720-6,880 and 24,080 mg/m³) from GD7 to GD16 (GD1 was the copulation day) (Haskell Laboratory 1997d). The animals were sacrificed on GD22 and examined grossly. Types of implants were counted and their relative positions were recorded. Live fetuses were weighed, sexed and examined for external, visceral and skeletal alterations.

Findings were limited to the dams and consisted in:

- a slight but significant decrease in implantation number with the number of corpora lutea unchanged compared with controls. This finding was consistent with slight pre-implantation losses and can be considered as not treatment-related since there was no treatment during the pre-implantation period. Moreover, this effect was not seen in the 2-generation study in rats at the same doses,
- statistically significant reductions in overall body weight gain and food consumption throughout the treatment period,
- a diminished response or no response of the animals to a sound stimulus while in the chamber during exposure and at 2,000 ppm and higher.

For this study, a NOAEL of 500 ppm (1,720 mg/m³) can be assumed for dams whereas a NOAEL of 7,000 ppm (24,080 mg/m³) can be assumed for pups considering the lack of toxic effects noticed.

Rabbits

A pilot inhalation developmental study of cyclohexane in rabbits was performed by Haskell (1997a). Four groups of eight pregnant New Zealand White rabbits were exposed whole-body to concentrations of cyclohexane varying between 0 and 7,000 ppm (0-500-2,000-7,000 ppm (0-1,720-6,880-24,080 mg/m³)) from Gestational Day 7 to 19 (GD0 was the copulation day). Dams were sacrificed on GD29 and examined for gross pathologies; implantations and resorptions were counted and their relative positions recorded; foetuses were weighed and examined externally for alterations. This study was performed according to EPA and OECD GLP.

The only effect observed was a slight statistical increase in the early resorptions at 7,000 ppm. However, this increase was in the limits of historical controls, the negative controls of this study were very low and this effect was not seen in the full study (see below). This effect can be considered as a result of biological variation (this must be compared with other studies and more complete studies).

This study was performed to serve as a pilot study for a more wide-ranging study on rabbits (Haskell Laboratory, 1997b) with the same experimental procedure. Four groups of 20 animals were exposed whole-body to the same concentrations levels (0-500-2,000-7,000 ppm (0-1,720-6,880-24,080 mg/m³)) from GD6 to GD18 (GD0 was the copulation day). Dams were euthanased on GD29 and examined for gross pathologies. Types of implants were counted (live/dead foetuses, resorptions) and their relative position recorded. Live foetuses were weighed, sexed and examined for external, visceral and skeletal alterations.

The only maternal effects reported was a slight decrease in the number of corpora lutea at doses of 2,000 and 7,000 ppm, but these variations were within historical control ranges. Moreover, these abnormalities occurred before the beginning of the treatment and cannot be related to cyclohexane. No systemic toxicity due to cyclohexane exposure was observed. No effects were observed on foetuses. For this study a NOAEL of 7,000 ppm (24,080 mg/m³) can be assumed for developmental effects in rabbits, both in dams and pups.

4.1.2.9.3 Summary of toxicity for reproduction

In the 2-generation study, no effect was seen on reproductive parameters. A slight decrease of the pups body weight was observed at 7,000 ppm, this decrease being accompanied by slight maternal toxicity. A NOAEL of 2,000 ppm (6,880 mg/m³) can be determined for pups whereas a NOAEL of 500 ppm (1,720 mg/m³) can be derived for maternal toxicity.

No toxic effect was observed in the foetuses in two developmental studies performed in rats and in rabbits. Toxic effects were noted in the dams and were consistent with those observed in the other studies (narcotic effects). The highest dose tested in these studies (7,000 ppm (24,080 mg/m³)) can be considered to be the NOAEL for foetuses with a NOAEL of 500 ppm (1,720 mg/m³) for dams.

Table 4.18 Summary of reproductive toxicity studies

Fertility			
Species	Administration protocol	Objectives of the tests	NOAEL
Rat	inh. 90 d	two-generation study	dams: 500 ppm (1,720 mg/m ³) pups: 2,000 ppm (6,880 mg/m ³)
Developmental toxicity study			
Species	Administration protocol	Objectives of the tests	NOAEL
Rat	inh. gestational day 7-16		dams: 3,000 ppm (10,320 mg/m ³) pups: > 3,000 ppm (10,320 mg/m ³)
Rat	inh. gestational day 7-16		dams: 500 ppm (1,720 mg/m ³) pups: > 7,000 ppm (24,080 mg/m ³)
Rabbit	inh. gestational day 7-19		dams: 7,000 ppm (24,080 mg/m ³) pups: > 7,000 ppm (24,080 mg/m ³)

4.1.3 Risk characterisation

4.1.3.1 General aspects

Cyclohexane has low acute toxicity properties via all routes. For neurotoxicity, narcotic properties and motor activity changes were demonstrated in a rat study leading to a NOAEL of 500 ppm (1,720 mg/m³). This value is based on transient and reversible effects after exposure to cyclohexane and could be considered as acute effects. In a recent study performed on human volunteers, cyclohexane was administered at doses of 25 and 250 ppm during a single 4-hour exposure. No neurobehavioral effect could be seen in any volunteer at each dose. The value of 250 ppm (860 mg/m³) will be taken into account in the risk characterisation for neurobehavioral toxicity.

Skin irritation is observed after repeated exposures. This is due to the defatting properties of cyclohexane. No sensitising properties can be anticipated.

After repeated dose exposure via inhalation route, the systemic effects observed in both mice and rats during the 28-day and 90-day studies, were limited to increase in absolute and relative liver weight, increases in mitotic index figures and centrolobular hypertrophy, leading to a NOAEL of 2,000 ppm (6,880 mg/m³). This is a very conservative value and this will be taken into account in the risk characterisation.

In an older study a NOAEL of 425 ppm (1,462 mg/m³) was recorded for systemic effects (Treon, 1943), but due to its limitations this value has not been taken into account.

No studies have been carried out to assess the sub-chronic effects via oral route and only one by dermal route, which does not allow the determination of a NOAEL by this route.

Cyclohexane is not genotoxic *in vitro* or *in vivo* and the only carcinogenic study available allows no conclusion to be drawn concerning its carcinogenic properties. However, a low carcinogenic potential can be expected.

Cyclohexane is not a developmental toxicant for rats and rabbits.

In the 2-generation study, no effects on fertility was reported and only small decreases in pups body weight were observed at 7,000 ppm (24,080 mg/m³) in the presence of maternal toxicity. This end-point will not be taken into account in the risk characterisation.

4.1.3.2 Workers

Occupational exposures mainly occur by inhalation and dermal routes. Oral exposure is not expected to be a direct route of exposure under normal occupational conditions. As cyclohexane is a volatile substance, the inhalation route is the most important to consider. Compared to inhalation exposure, dermal exposure is negligible and is therefore not a matter for concern.

For risk characterisation, we will consider four different occupational scenarios, as previously described (Section 4.1.1.1.2).

Acute toxicity (lethal effects)

No concern: **conclusion (ii)** for all scenarios.

Acute toxicity (neurologic effects)

Production (Scenario 1)

According to the available data measured on production sites the mean representative atmospheric concentration is less than 3 ppm (maximum value 20 ppm). During certain operations (tank filling, etc.), short-duration exposure can be as high as 30 ppm. These actual values will be used for risk evaluation as they are higher than the EASE exposure estimation (0-0.1 ppm).

In this scenario the range of dermal exposure was estimated to be 0-0.1 mg/cm²/day (EASE model). When both hands are exposed (840 cm²), and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be in the range 0 mg/kg/day to 0.06 mg/kg/day for a 70-kg worker ((0.1 · 840/70) · 5%). This value is overestimated as the difference between dermal absorption in rat and humans and the evaporation of cyclohexane from the skin have not been taken into account. This result clearly indicates that dermal exposure is negligible compared to inhalation exposure which corresponds to an internal dose of 10 mg/kg/day (70 mg/m³ · 10 m³/70 kg).

In this case, considering only inhalation exposure, the MOSs (NOAEL/exposure ratio) are acceptable for neurobehavioral effects: 250 ppm/20 ppm = 12.5 (general case) or 250 ppm/30 ppm = 8.3 (acute exposure): **conclusion (ii)**.

During industrial use as intermediate or solvent in the chemical industry (Scenario 2)

In this case the atmospheric concentrations at the workplace are considered to be the same as in Scenario 1. Dermal exposure was estimated by the EASE model, leading to the same range as in Scenario 1. The same conclusion as in Scenario 1 is applicable: **conclusion (ii)**.

During formulation and industrial use of cyclohexane containing products (Scenario 3)

The measured data show that the exposure level covering this use is often lower than the limit value. It sometimes gets close to the limit value of 300 ppm (current value in most countries), and in some cases may go as high as 954 ppm when working practices are very bad. The EASE model predicts an exposure in the range 10-500 ppm, which is consistent with most of the measured data. For risk characterisation, it seems reasonable to take into account the exposure value of 300 ppm (1,032 mg/m³).

The range of dermal exposure was estimated to be 0.03-0.3 mg/cm²/day (EASE model). When both hands are exposed (840 cm²) and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be in the range of 0.02-0.2 mg/kg/day for a 70-kg worker ((0.03 · 840/70) · 5% and (0.3 · 840/70) · 5%). This value is overestimated as the difference between dermal absorption in rats and humans has not been taken into account. This result clearly indicates that dermal exposure is negligible compared to inhalation exposure which corresponds to an internal dose of 147 mg/kg/day (1,032 mg/m³ · 10 m³/70 kg).

MOSs are 250 ppm/300 ppm = 0.83 for neurobehavioral toxicity. It should be emphasised that the exposure may be much higher. Taking into consideration that the observed effects at the doses above the NOAELs are weak and observed in a human study, it is not necessary to have a

high safety factor. However the ratio 0.83 is insufficient for the protection of workers. In this case, there is a need for limiting the risks: **conclusion (iii)**.

Use as of cyclohexane containing products in craft industries (Scenario 4)

No measured exposure data are available. The EASE model predicts an inhalation exposure in the range of 200 to 1,000 ppm (680-3,440 mg/m³).

The range of dermal exposure was estimated to be 1.5-4.5 mg/cm²/day (EASE model). When both hands are exposed (840 cm²) and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be in the range of 0.9-2.7 mg/kg/day for a 70-kg worker ((1.5·840/70)·5% and (4.5·840/70)·5%). This value is overestimated, as the difference between dermal absorption in rats and humans has not been taken into account. This result clearly indicates that dermal exposure is negligible compared to inhalation exposure which corresponds to an internal dose of 98-491 mg/kg/day (688 mg/m³·10 m³/70 kg and 3,440 mg/m³·10 m³/70 kg).

The NOAEL/exposure ratio, 250 ppm/1,000 ppm = 0.25 is not acceptable for neurobehavioral effects. There is a need for limiting the risks: **conclusion (iii)**.

Irritation

Irritation is an issue of concern for cyclohexane. It could be argued that this hazard should receive a conclusion (iii) to take into account all conceivable situations where controls fail or are not properly applied.

However, risk reduction measures already exist in the EU regulation, including classification and labelling, safety data sheet and the thereby invoked engineering controls and personal protective equipments.

Consequently, there is no need for risk reduction measures beyond those which should already be applied: **conclusion (ii)** for all scenarios.

Sensitisation

No concern: **conclusion (ii)** for all scenarios.

Hepatic effects (chronic effects)

Production (Scenario 1)

According to available data measured on production sites, the mean representative atmospheric concentration is less than 3 ppm (maximum value 20 ppm). The values of 20 ppm (worst case) will be used for chronic risk evaluation, as they are higher than EASE exposure estimation (0-0.1 ppm).

In this scenario, the range of dermal exposure was estimated to be 0-0.1 mg/cm²/day (EASE model). When both hands are exposed (840 cm²), and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be between 0 and 0.06 mg/kg/day for a 70-kg worker ((0.1·840/70)·5%). This value is overestimated as the difference between dermal absorption in rats and humans and the evaporation of cyclohexane from the skin have not been taken into account. This result clearly indicates that dermal exposure

is negligible compared to inhalation exposure which corresponds to an internal dose of 10 mg/kg/day ($70 \text{ mg/m}^3 \cdot 10 \text{ m}^3/70 \text{ kg}$).

In this case, considering only inhalation exposure, the MOSs (NOAEL/exposure ratio) is acceptable for hepatic effects $2,000 \text{ ppm}/20 \text{ ppm} = 100$: **conclusion (ii)**.

During industrial use as an intermediate or a solvent in the chemical industry (Scenario 2)

In this case the atmospheric concentrations at the workplace are considered to be the same as in Scenario 1. Dermal exposure was estimated by the EASE model leading to the same range as in Scenario 1. The same conclusion as in Scenario 1 is applicable: **conclusion (ii)**.

During formulation and industrial use of cyclohexane containing products (Scenario 3)

The measured data show that the exposure level covering this use is often lower than the limit value. It sometimes get close to the limit value of 300 ppm (current value in most countries), and in some cases it may go as high as 954 ppm when working practices are very bad. The EASE model predicts an exposure in the range 10-500 ppm, which is consistent with most of the measured data. For risk characterisation, it seems reasonable to take into account the exposure value of 300 ppm ($1,032 \text{ mg/m}^3$).

The range of dermal exposure was estimated to be 0.03-0.3 mg/cm²/day (EASE model). When both hands are exposed (840 cm^2), and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be between 0.02 and 0.2 mg/kg/day for a 70-kg worker ($(0.03 \cdot 840/70) \cdot 5\%$ and $(0.3 \cdot 840/70) \cdot 5\%$). This value is overestimated as the difference between dermal absorption in rats and humans has not been taken into account. This result clearly indicates that dermal exposure is negligible compared to inhalation exposure, which corresponds to an internal dose of 147 mg/kg/day ($1,032 \text{ mg/m}^3 \cdot 10 \text{ m}^3/70 \text{ kg}$).

MOSs are $2,000 \text{ ppm} / 300 \text{ ppm} = 6.66$ for chronic systemic toxicity. It should be emphasised that the exposure may be much higher. Taking into consideration that the observed effects at the doses above the NOAELs are weak, it is not necessary to have a high safety factor. However the ratio 6.66 is insufficient for the protection of workers. In this case, there is a need for limiting the risks: **conclusion (iii)**.

Use as of cyclohexane containing products among craftsmen (Scenario 4)

No measured exposure data are available. The EASE model predicts an inhalation exposure of between 200 and 1,000 ppm ($688\text{-}3,440 \text{ mg/m}^3$).

The range of dermal exposure was estimated to be 1.5-4.5 mg/cm²/day (EASE model). When both hands are exposed (840 cm^2), and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal absorption by this route would be between 0.9 and 2.7 mg/kg/day for a 70-kg worker ($(1.5 \cdot 840/70) \cdot 5\%$ and $(4.5 \cdot 840/70) \cdot 5\%$). This value is overestimated as the difference between dermal absorption in rats and humans has not been taken into account. This result clearly indicates that dermal exposure is negligible compared to inhalation exposure which corresponds to an internal dose of 98-491 mg/kg/day ($688 \text{ mg/m}^3 \cdot 10 \text{ m}^3/70 \text{ kg}$ and $3,440 \text{ mg/m}^3 \cdot 10 \text{ m}^3/70 \text{ kg}$).

The NOAEL/exposure ratio, $2,000 \text{ ppm} / 1,000 \text{ ppm} = 2$ is not acceptable for chronic systemic effects. There is a need for limiting the risks: **conclusion (iii)**.

Mutagenicity

No concern: **conclusion (ii)** for all scenarios.

Carcinogenicity

No concern: **conclusion (ii)** for all scenarios.

Reproductive toxicity

No concern: **conclusion (ii)** for all scenarios.

Conclusion of the risk assessment for workers

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

This conclusion is reached because of concerns for acute toxicity (neurobehavioural effects) and general systemic toxicity (hepatic effects) as a consequence of inhalation exposure arising from formulation and industrial use of products containing the substance as well as from use of products containing the substance in craft industries.

4.1.3.3 Consumers

No quantitative data could be obtained for the evaluation of consumer exposure, either from the chemical industry or through a review of the literature. However, it is highly probable that consumer exposure mainly occurs by inhalation.

The use of adhesives containing cyclohexane is by far the main source of consumer exposure and this assessment is focused on exposure during carpet laying, which is expected to be the highest exposure.

Inhalation and dermal exposure of consumers has been considered as similar to the exposure of professional carpet layers. The EASE model predicts a maximum airborne concentration of 1,000 ppm (3,440 mg/m³) and an external dermal exposure of between 1.5-4.5 mg/cm²/day.

Assuming an eight hour/event and an inhalation rate of 10m³/8 hours, the inhalation exposure corresponds to an internal dose of 573 mg/kg/day (3,440 mg/m³ · 10 m³/60 kg).

When both hands are exposed (840 cm²) and given the absorption of cyclohexane of 5% in liquid phase (in rats), the internal exposure by this route would be between 1 and 3.1 mg/kg/day for a 60-kg consumer (1.5 · 840/60) · 5% and (4.5 · 840/60) · 5%. This value is overestimated as the difference between dermal absorption in rats and humans has not been taken into account. This result clearly indicates that dermal exposure is negligible compared to the inhalation exposure which corresponds to an internal dose of 573 mg/kg/day

Based on the frequency of use, only acute effects are considered relevant for the risk characterisation. The NOAEL/exposure ratio, 250 ppm / 1,000 ppm = 0.25 is not acceptable for neurobehavioral effects. There is a need for limiting the risks: **conclusion (iii)**.

4.1.3.3 Humans exposed via the environment

The highest estimated "worst-case" indirect exposure of humans is due to air concentration in the vicinity of sites using cyclohexane as a solvent for chemical production processes, i.e. ca. 0.521 mg/m^3 (ca. 0.15 ppm). Compared with the lowest available NOAEL of 250 ppm (neurotoxicity) an MOS of $250/0.15 = 1,666$ can be derived.

In addition to the indirect exposure due to the industrial use of cyclohexane, humans are exposed through car exhausts. As seen above, the measured concentrations of cyclohexane in the vicinity of busy roads are approximately $1\text{-}10 \text{ }\mu\text{g/m}^3$ i.e. 0.0003–0.003 ppm. An MOS of 83,333 can be derived.

No risk can be derived from the exposure to humans via the environment: **conclusion (ii)**.

4.1.3.4 Combined Exposure

In the case of combined exposure, the highest potential uptake is likely to be present during occupational exposure. Consumer exposure and indirect exposure via environment can be considered as negligible.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

Given the fact that cyclohexane is a volatile, highly-flammable liquid, its uncontrolled use can rapidly lead to a dangerous concentration building up in the air.

In industry, whether it is during manufacture or industrial use, effective controlled measures are taken in accordance with current regulations (ventilation of work areas, regulations regarding electrical equipment).

Such measures are not always taken during use by craftsmen. However, it should be noted that in this case, cyclohexane is never used alone but is mixed with other solvents, many of which are also flammable.

4.2.1.2 Consumers

Consumer exposure cannot actually be evaluated, as few data could be obtained on the cyclohexane content of commercial products, neither from the chemical industry nor through a review of the literature.

Consumers are exposed to the flammability hazard in the case of its use as a solvent in do-it-yourself products, not only because of cyclohexane but also because of the other solvents combined with it. The only preventative measures possible are the precautions to be taken by the user themselves, which include not smoking when using it and using it away from flames or sparkles in a well-ventilated place; this information must be on a label directly on the container.

4.2.1.3 Humans exposed via the environment

Not applicable.

4.2.2 Effects assessment: Hazard assessment

4.2.2.1 Explosivity

Because of the structure of its molecule (no reactive groups present), cyclohexane does not need to be tested; it is unlikely to have explosive properties.

4.2.2.2 Flammability

Cyclohexane is a highly flammable liquid (flash point: -20°C) with a self-ignition temperature of 260°C . It is a volatile liquid (vapour pressure 13 KPa at 20°C). The vapours can form flammable

and explosive mixtures with air within the range of 1.33% to 8.35 volume %. Cyclohexane is a static accumulator.

4.2.2.3 Oxidising potential

The test method (OECD-EEC) is not applicable to liquid substances.

Because of the structure of its molecule, cyclohexane is unlikely to have oxidising properties.

4.2.3 Risk characterisation

Regarding its physico-chemical properties, flammability is the only property of concern for cyclohexane since it is a volatile liquid which is highly flammable and can form explosive mixtures with air.

In production and in occupational use, the flammability risk is not of concern provided adequate safety measures are taken. Information is provided on the label and in the safety data sheet.

Concerning use by consumers, information about the flammability risk and precautionary measures must be given by a label on the containers: in the EU, symbol, risk phrases and safety phrases are used for the labelling of highly flammable substances and preparations (mixtures), cf. Section 1 - classification.

Conclusion (ii).

5 RESULTS

5.1 ENVIRONMENT

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

This conclusion applies to all environmental compartments and all identified uses of cyclohexane: use as a chemical intermediate, use as a solvent in chemical production processes and use as a solvent in adhesives and coatings.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

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

This conclusion is reached because of concerns for acute toxicity (neurobehavioural effects) and general systemic toxicity (hepatic effects) as a consequence of inhalation exposure arising from formulation and industrial use of products containing the substance as well as from use of products containing the substance in craft industries.

5.2.1.2 Consumers

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

This conclusion is reached because of concerns for acute toxicity (neurobehavioural effects) as a consequence of exposure arising from use of products containing the substance.

5.2.1.3 Humans exposed via the environment

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

5.2.2 Human health (risks from physico-chemical properties)

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

6

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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
BEEL	Biological Equivalent Exposure Limits
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / <i>Bw</i> , <i>bw</i>
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II 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
CHO	Chinese Hamster Ovary
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 / <i>dw</i>
DCB	DNA Cell Binding
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DMBA	dimethylbenzanthracene
DMSO	dimethyl sulfoxide
DNA	DeoxyriboNucleic Acid

DNCB	1-chloro-2,4-dinitrobenzene
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II 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 II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
FOB	Functional Observational Battery
foc	Organic carbon factor (compartment depending)
GCP	Good Clinical Practices
GD	Gestational Day
GLP	Good Laboratory Practice
HB	Haemoglobin
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HEG	Homogeneous Exposure Group
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission

HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
Ht	Haematocrit
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
ITI	Inter-Trial Interval
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
LDH	Lactate dehydrogenase
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
MA	Motor Activity
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MDL	Motor Distal Latency
MITI	Ministry of International Trade and Industry, Japan
MMS	Methyl methanesulfonate
MNCV	Maximum Nerve Conduction Velocity
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 II of Directive 67/548/EEC)
NAEL	No Adverse Effect Level
NCV	Nerve Conduction Velocities
NDEA	N-nitrosodiethylamine
NDMA	N-nitrosodimethylamine
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NP	NeuroPathology
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
PPD	Post Partum Day
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
RCB	Red Blood Cells

RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SCOB	Schedule-Controlled Operant Behaviour
SDH	Sorbitol dehydrogenase
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNCVd	Distal conduction velocity of sensory fibers
SNCVp	Proximal conduction velocity of sensory fibers
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STEL	Short Term Exposure Limit
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
TPA	12-O-tetradecanoylphorbol-13-acetate
TWA	Time Weighed Average
ThOD	Theoretical 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 II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Annex 1

TNO studies: Vmax and Km for cyclohexane metabolism in rats compared with a physiologically based pharmacokinetic (PbPk) simulation model in cross species extrapolation.

Groups of 3 male WAG/RijCR/BR rats (Wistar "Genetic epileptic rat") were exposed to concentrations of 100-600-4,000 and 24,000 mg/m³ (30 to 7,000 ppm) of cyclohexane. Actual concentrations in the test atmosphere were monitored at five-minute intervals. In order to evaluate fur adsorption, a further group of rat was sacrificed just before exposure and submitted to the same experimental conditions at concentration of 6,000 mg/m³.

Experimental results were compared with a five-compartment PbPk model. A similar four-compartment model was first described by Krishnan and Andersen (1994) for other lipophilic volatile organics. However, since CNS was considered to be a target organ, a fifth compartment has been added (brain compartment). This model is described in figure 1. Data from other TNO studies and available literature were included in the model (see Sections 4.1.2.1 and 4.1.2.2).

Cyclohexane was assumed to be eliminated through saturable biotransformation in the liver so calculation of the metabolic clearance was based on: $dA_1/dt = V_{max} \cdot C_{vl} / (K_m + C_{vl})$.

Experimental results, verified with modelisation were:

- Vmax c = 5 mg/hr (normalised to 1 kg of body weight)
- Km = 0.1 mg/l

The physiological and biological parameters used in the model are summarised in **Table A1**.

According to this model, it is estimated that an exposure level of about 1,200 ppm would not result in acute neurotoxicity in human. This result is presented in a brief poster (Hissink, 1999) without details about the method and the initial rat NOAEL chosen to extrapolate to human. It is probable that this is a rat to human extrapolation based on the TNO study about neurotoxicity (TNO, 1998b) in which the NOAEL was 2,000 ppm in rats based on effects seen at 8,000 ppm. For the same study, we have preferred to take a more conservative NOAEL of 400 ppm because of the slight effects seen at 2,000 ppm. In this case the calculated NOAEL of 1,200 ppm in human can be considered to be a LOAEL for us.

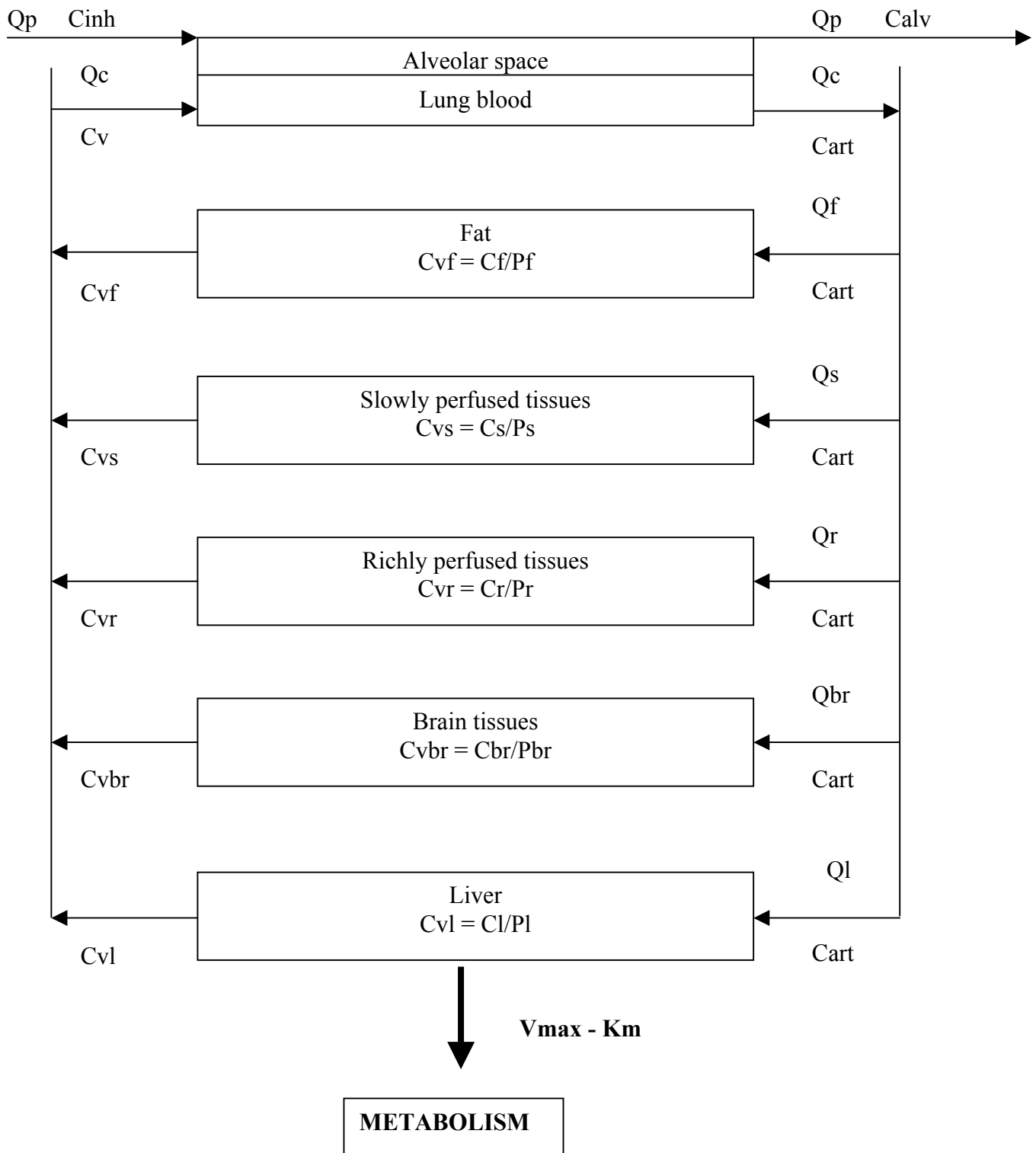
Moreover in a study performed with human volunteers (TNO, 1998c), a tested dose of 250 ppm during 4 hours did not show any effects. This NOAEL of 250 ppm is also consistent with the LOAEL of 1,200 ppm.

This NOAEL of 250 ppm was chosen keeping in mind this value is very conservative because it was the highest dose tested and that no effects were seen. This was obviously taken into account for the calculation of acceptable MOSs concerning this endpoint.

Table A1 Physiological, biochemical parameters and partition coefficients used in the PbPk model for cyclohexane

Parameter	Rat	Human
Flows (percentage of total cardiac output)		
Liver	25	25
Fat	9	5
Brain	1.2	14
Richly perfused	49.8	31
Slowly perfused	15	25
Volumes (percentage of body weight)		
Liver	4	2.6
Fat	7	19
Brain	0.72	2
Richly perfused	4.28	3
Slowly perfused	75	62
Partition coefficients		
Blood/air	1.3	1.3
Richly perfused/blood	7.6	7.6
Slowly perfused/blood	3.92	3.92
Fat/blood	180	180
Brain/blood	7.6	7.6
Liver/blood	7.6	7.6
Biochemical parameters		
Vmax (mg/hr/kg)	5	5
Km (mg/l)	0.1	0.1

Figure 1 PkPb model



European Commission

**EUR 21015 EN European Union Risk Assessment Report
cyclohexane, Volume 41**

Editors: S.J. Munn, R. Allanou, K. Aschberger, F. Berthault, J. de Bruijn, C. Musset, S. O'Connor, S. Pakalin, A. Paya-Perez, G. Pellegrini, S. Scheer, S. Vegro.

Luxembourg: Office for Official Publications of the European Communities

2004 – VIII pp., 108 pp. – 17.0 x 24.0 cm

Environment and quality of life series

The report provides the comprehensive risk assessment of the substance cyclohexane. It has been prepared by France in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The environmental risk assessment for cyclohexane concludes that there is no concern for the aquatic ecosystem, the terrestrial ecosystem, the atmosphere or for microorganisms in the sewage treatment plant as well as for secondary poisoning.

The human health risk assessment for cyclohexane concludes that there is concern for workers and consumers. For humans exposed via the environment the risk assessment concludes that risks are not expected.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commissions committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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European Commission – Joint Research Centre
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European Union Risk Assessment Report

cyclohexane

CAS No: 110-82-7 EINECS No: 203-806-2

Series: 1st Priority List Volume: 41