RISK ASSESSMENT

2-Ethoxyethanol

Human Health only

CAS-No.: 110-80-5

EINECS-No.: 203-804-1

Draft of 21.11.2008

Information on the rapporteur

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The first draft of the Human Health Section of the Comprehensive Risk Assessment Report 2-Ethoxyethanol, a substance chosen from the EU 2nd Priority List in 1995.

CONTENTS

0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 110-80-5

EINECS No. 203-804-1

IUPAC Name 2-Ethoxyethanol

Overall results of the risk assessment:

( ) i) There is need for further information and/or testing

(x) 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

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

Summary of conclusions:

Environment

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

Based on the available data, 2-ethoxyethanol represents no risk to the environment resulting from production, processing, formulation and use.

Workers

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

Concern is derived for developmental toxicity. The corresponding critical exposure level of 0.72 mg/m^3 for inhalation resp. 0.18 mg/kg/day for dermal contact are threefoldlower, than the exposure values of scenario 1 (production and further processing in the large scale industry) for inhalation (3 mg/m^3) and dermal contact (0.3 mg/kg/day).

Consumers

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

Humans exposed via the environment

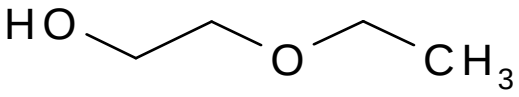
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

1 GENERAL SUBSTANCE INFORMATION

Identification of the substance

CAS-No.:	110-80-5
EINECS-No.:	203-804-1
IUPAC Name	2-ethoxyethanol
Synonyms:	ethylglycol 2-EE ethylene glycol monoethyl ether
Molecular weight:	90.1 g/mol
Empirical formula:	$C_4H_{10}O_2$

Structural formula:



Purity/impurities, additives

Purity:	> 99 % w/w
Impurity:	< 0.005 % w/w acetic acid
	$\leq$ 0.2 % w/w water
Additives:	< 0.012 % w/w 2,6-di-tert-butyl-p-cresol
	2-aminoethanol
	function: inhibition of peroxide formation

Physico-chemical properties

2-Ethoxyethanol is a colourless liquid at 20 °C at room temperature and normal pressure. Data on the physical and chemical properties are given in table 1.1.

Table 1.1: Physico-chemical properties

Melting point	< - 80 °C	Ullmann, 1978
Boiling point	132 - 137 °C at 1013hPa	Ullmann, 1978
Relative density	0.930 at 20 °C	Ullmann, 1978
Vapour pressure	5.3 hPa at 20 °C	Kirk-Othmer, 1980
Surface tension	69.5 mN/m at 25 °C [1)]	Union Carbide, 1998
Water solubility	miscible in each ratio at 20 °C	Kirk-Othmer, 1980
Partition coefficient	log Pow –0.54 to –0.10 [2)]	Dearden & Bresnen, 1988
Flash point	40 °C (closed cup)	Chemsafe, 1996
Flammability	flammable [3)]	Chemsafe, 1996
Ignition temperature	235 °C	Chemsafe, 1996
Explosive properties	not explosive [4)]	Chemsafe, 1996
Oxidising properties	no oxidising properties [5)]	Chemsafe, 1996
Henry's law constant	0.003 Pa * m³ * mol-1	Howard, Meylan; SRC 1993

1) Ring method

2) In the following risk assessment report a log Pow of – 0.43 is used

3) Test A.10 not conducted (substance is a liquid)
Test A.12 and A.13 not conducted because of structural reasons

4) No test conducted because of structural reasons

5) No test conducted because of structural reasons

Classification

Classification according to Annex I of directive 67/548/EEC:

Reprotox. Cat. 2,	
R 60	may impair fertility
R 61	may cause harm to unborn child
T	toxic
Xn	harmful
R 10	Flammable
R 20/21/22	harmful by inhalation, in contact with skin and if swallowed

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION/ IMPORT

According to the current information (INEOS 2006) only one production site (site A) of 2-ethoxyethanol is remaining in the EU. There is no known import from outside of the EU. No information is available on possible exports of 2-ethoxyethanol.

The submitted information on production in the EU indicates varying volumes for the last years production with no clear trend. Hence, the data from the last 6 years (2000- 2005) were averaged resulting in a yearly volume of approximately 1000 t/a of 2-ethoxyethanol. This volume is used for the risk assessment. The detailed production volumes are shown in the following table:

Table 2.1: Detailed production volumes

2000	950 t/a
2001	1384 t/a
2002	1360 t/a
2003	1401 t/a
2004	485 t/a
2005	520 t/a

2.2 PROCESSING / APPLICATION (CATEGORIES OF USE, AMOUNTS)

The main proportion of 2-ethoxyethanol is processed to intermediates such as the 2-ethoxyethanol tert. butyl ether in chemical industry. The smaller part is industrially used as a solvent.

2-ethoxyethanol was chosen for risk assessment because of the previous high production volume. It was widely used in open systems, such as paints for privat use, in surface treatment of metals and in repair industry. Besides the industrial use as intermediate and solvent, 2-ethoxyethanol was used for the formulation of paints, lacquers, varnishes and printing inks.

Based on the latest information (INEOS 2006), there is no remaining wide dispersive use of 2-ethoxyethanol outside the chemical industry. The current use pattern is as follows:

Table 2.2: Current use pattern

Main category (MC)	Industrial category (IC)	Use category (UC)	Mass balance [in % of use]
Non-dispersive use (1)	Chemical industry (3)	Intermediate (33)	80
Non-dispersive use (1)	Chemical industry (3)	Solvent (48)	20

According to BUA (1995), information provided by the lead company (INEOS 1996) an additional use for 2-ethoxyethanol as anti-freeze additive for aviation fuels and for clearing runways is obsolete now and to current.

According to the Danish Product Register the total annual use of 2-ethoxyethanol in 1996 exclusively in Denmark, was exceeding 2000 t/a. Currently, information about the use amounts in Norway, Sweden, Denmark and Finland are listed at SPIN (Substances in Preparations in Nordic Countries). The latest information given there is a total amount of 209.3 tonnes in 2004. Further, 2-ethoxyethanol was reported as solvent in cleaning agents/disinfectants and cosmetics for personal/domestic use. Currently, there is no personal/domestic use anymore due to a voluntary program of industry. This programme was initiated due to the toxic effects on reproduction (R 60/ R 61 labelling).

According to the German Washing and Cleansing Agents Act information on ingredients and expected production quantities is supplied to the German Federal Environmental Agency. A use of 75 t 2-ethoxyethanol / a for the application as industrial solvent is registered there (UBA 2006).

3 ENVIRONMENT

3.1.1 General discussion

Release into the environment

During production, processing (use as an intermediate), and the use as solvent, 2-ethoxy-ethanol is expected to be released into the environment via waste water and exhaust air.

Specific release data were not submitted by IND for downstream uses.Therefore a generic approach has been chosen for downstream uses. Specific information on manufacturing process and release data for the remaining production at site A was given as follows:

Manufacturing Process (site A):

2-ethoxyethanol is the reaction product of ethanol and ethylene oxide in the presence of a base catalyst.

The catalyst is made up in a prebatch with ethanol and fed in the ethanol stream to the pipe reactor. Subsequently ethylene oxide is added in a static mixer. After preheating the mixture up to the reaction temperature it flows to the pipe reactor where the reaction proceeds in a range of 150-200°C and 15 bar. The reaction takes place in an excess amount of ethanol.

Under these conditions the reaction rates in the liquid phase are very fast resulting in a very fast decline in free ethylene oxide. Finally, in a tank reactor sufficient residence time is provided to achieve complete ethylene oxide conversion.

The product stream from the 2-ethoxyethanol tank reactor is fed to an alcohol removal column where the remaining ethanol is removed from the crude glycol ether mixture. The ethanol is recycled back to the pipe reactor. In order to recover the individual glycol ether products at high purity level the bottom productstream (= the crude glycol ether) flows to the purification section which consists of 2 columns called the 'cellostill ' and the 'carbistill '.

In the 'cellostill 'the topstream is highly purified 2-ethoxyethanol.

The bottom stream of this columns further fractionated in the 'carbistill' resulting in 2-ethoxyethanol as the top stream. The bottom stream of the last vacuum column contains the heavier glycol ethers.

All these glycol ether products are cooled and routed to the storage where they are stored under a Nitrogen blanket.

Release during production

Emissions to air:

The whole process as described above is a closed system. Only the vents of the two distillation columns are emitting to the air. The average mass loss is approx. 0.01 kg/t 2-ethoxyethanol produced. Breathing losses of tanks are minimised by storing the end products under a Nitrogen blanket.

Emissions to water:

Average product loss via the aqueous effluent resulting from the steam condensate from the two columns is 2 kg TOC/t 2-ethoxyethanol produced. This effluent, mixed up with other streams is bio-treated with high efficiency in the central waste water treatment plant (WWTP). According to producer information, no 2-ethoxyethanol has ever been detected in the WWTP-effluent (detection limit and further details not submitted).

By-products/ wastes:

The bottomstream of the 'carbistill' is consisting of higher glycol ethers (approx. 20 kg/t 2-ethoxyethanol produced) which are incinerated with energy recuperation.

Regarding downstream uses, 2-ethoxyethanol is no longer included in private consumer goods. For industrial plants it can be assumed that to a relevant percentage equipment for extracting the solvent from the exhaust air is installed. Therefore, the calculated releases into the atmosphere in the following tables represent the worst case assumption with no recuperation of solvent from exhaust air, using the A- and B – tables of TGD.

Degradation

Biodegradation

The biodegradation of 2-ethoxyethanol has been determined according to two OECD standard tests. In the Modified OECD Screening Test (OECD 301 E) (Hüls AG, 1995a) performed in 1979 [Reliability 1, according to Klimisch, 1997], 944 mg of 2-ethoxyethanol were added to a mineral medium which was aerated and inoculated at a temperature of 20 °C. The inoculum used was taken from a municipal waste water treatment plant (WWTP) that is considered to be not adapted (Hüls, personal communication, 1999). It was stated that industrial wastewater was never introduced into the municipal WWTP, but treated in a pilot treatment plant and a stabilisation pond before discharge into the river Lippe until 1981. Then the industrial WWTP at the processing site was constructed. 2-ethoxyethanol was degraded at 100 % (measured as DOC) within a period of 14 days. The pass level for ready biodegradability of 70 % within the 10 day window was achieved.

In a Zahn Wellens Test (OECD 302 B) (Hüls, 1995b), Ethylglycol D was used (no further details). 650 mg/l was added to the mineral medium, the inoculum was from the same WWTP as indicated above. The dry weight of the activated sludge utilised was 1.2 g/l. The study was conducted at 20 - 22 °C in a static system. 2-ethoxyethanol was degraded at 100 % (measured as DOC) within a period of 9 days the pass level for inherent biodegradation was achieved [Reliability 1].

Another study was performed with 2-ethoxyethanol. In a MITI test (MITI, 1992), it was shown that at a concentration of 100 mg/l test substance, 63 - 83 % was degraded within 14 days (determined by BOD analysis). The inoculum concentration was 30 mg/l, the reference substance was aniline [Reliability 1].

Based on these results, 2-ethoxyethanol is classified as „readily biodegradable“.

Zahn and Wellens also determined in a static test system (inoculum dry weight: 1 g/l) at an initial concentration of 1000 mg/l a degradation rate of > 90 % after a 5 day incubation period. Following a lag phase of 3 days, the rate of biodegradation was 30 % COD per day (Zahn and Wellens, 1980) [Reliability 2].

Biodegradation tests were also performed according to APHA methods (American Public Health Association) (Price et al. 1974, Bridié et al. 1979b). Biological oxygen demand (BOD) of 2-ethoxyethanol was measured at concentrations of 3, 7 and 10 mg/l. The inoculum was domestic settled sewage (only detail in regard to the inoculum concentration: 3 ml in „half filled“ BOD bottles). After 20 days, 100 % of the test substance was degraded, with a 88 % degradation level at day 10. However, it is not apparent at which concentration the above results were attained (Price et al. 1974) [Reliability 2].

In the same publication, a second test system was used. The seed source was maintained by adding small amounts of settled raw wastewater every 3 to 4 days as substrate, seed bacteria and growth factors to seawater. With exception of the seed source, the biodegradation tests were performed in the same manner as in the freshwater tests. After 20 days, a degradation of 62 % was determined by BOD measurements. In 10 days, 42 % were degraded. However again, the initial test substance concentration is not known (Price et al. 1974) [Reliability 2].

With regard to the elimination in WWTP's, Kupferle (1991) reported elimination rates in two pilot plants fed with synthetic feed and 2-ethoxyethanol as primary carbon source at concentrations of 500 and 2500 mg/l. After 4 days, the removal of the substance was 81 % and 99 %, respectively. By DOC analysis, a degradation rate of 70 % and 86 % was determined after the same contact time. No 2-ethoxyethanol was detected in the off gas samples or in the wasted liquor. The more complete removal of the test substance at the higher concentration was explained by an improvement of the test system operations. Since 2-ethoxyethanol was already introduced to the test system during so-called acclimation processes, it is obvious that adaptation took place [Reliability 2].

Conclusion:

According to the standard test on ready biodegradation and further experimental results with high biodegradation rates, 2-ethoxyethanol is classified as readily biodegradable.

In addition, Kupferle (1991) determined high degradation levels using adapted inocula. With regard to the results obtained by Bridié et al. (1979b), it has been demonstrated that high biodegradation rates have been obtained with adapted and non-adapted sewage.

Since no tests for biodegradation in soil and sediment are available, degradation constants and half-lifes for soil and sediment are calculated based upon the results of the ready biodegradability test.

Hence, for the exposure estimation, the following rate constants for biodegradation of 2-ethoxyethanol were assumed according the TGD. Since the substance is considered as ready biodegradable based on test results, the derived half-life time in sediment is probably unrealistic in nature.

Table 3.1) Deagradation constants and half-lifes

Compartment	**degradation constant**	**Half life**
Waste water treatment plant	$kbio_{WWTP}$ = 1 h^{-1}	0.7 h
Aquatic environment	$kbio_{SW}$ = 0.047 d^{-1}	15 d
Soil	$kbio_{SOIL}$ = 0.023 d-1	30 d
Sediment	$kbio_{SED}$ = 0.0023 d^{-1}	300 d

(see Appendix A1 for calculation)

Photodegradation in air

An estimation of the half-life for the atmospheric reaction of 2-ethoxyethanol with hydroxyl radicals using the program AOP 1.87 yields a value of 22.2 h (24-h day, $5 \cdot 10^5$ OH/cm^3). An experimental study implemented in the same program yields a reaction rate constant of $15.4 \cdot 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1}, which corresponds to an experimental half-life of 25.0 h (Atkinson R., 1989).

Consequently, possibly emitted ethoxyethanol will be degraded rapidly in the air.

Hydrolysis and Photolysis

Experimental results about the hydrolysis of 2-ethoxyethanol are not available. Considering the chemical structure of 2-ethoxyethanol, hydrolysis is not to be expected. In addition, photolytic degradation in water is not expected, since no relevant absorption above a wavelength of 290 nm is expected for alcohols and ethers (Howard et al., 1993).

Distribution

With a Henry's law constant of 0.048 Pa m^3 mol^{-1} 2-ethoxyethanol is considered as moderate volatile. The classification of 'moderate volatility' is defined by being > 0.03 and < 100 Pa m^3 mol^{-1} (Thomas, 1982). Hence, the extent of volatilisation from surface waters can be neglected.

Since no experimental results about adsorption of 2-ethoxyethanol to soil are available, the estimation of the adsorption coefficients to soil, sediment and suspended matter are performed according to the TGD, using a log Pow of -0.43 and calculating a K_{OC} of 6.3 l/kg with an appropriate SAR-equation (see Appendix A1). The adsorption to sewage sludge is negligible (see table 3.4) and need therefore not be calculated.

Due to the classification schema by Blume and Ahlsdorf (1993) the adsorption of 2-ethoxyethanol is classified as 'very low'.

The combined results for the compartment-specific adsorption coefficients are summarised in the following table (the detailed calculations are given in appendix A1):

Table 3.2) Partition coefficients

Compartment	**Partition coefficient 2-ethoxyethanol**
Soil-water	$K_{p\text{-}soil}$ = 0.125 l/kg
Sediment-water	$K_{p\text{-}sed}$ = 0.313 l/kg
Suspended matter-water	$K_{p\text{-}susp}$ = 0.626 l/kg

The following theoretical distribution in the environment results from using the multimedia fugacity model EQC (Mackay Level I) and the physico-chemical properties given in chapter 1.

Table 3.3) Distribution of 2-ethoxyethanol

Compartment	**% 2-ethoxyethanol**
Air	0.97
Water	99.0
Soil/Sediment	0.03

Consequently, the hydrosphere is the target compartment for 2-ethoxyethanol regarding distribution in the environment.

Elimination in waste water treatment plants

Based on the physico-chemical properties of ethoxyethanol (log H = -1.32 log Pow = -0.43) and the estimated biodegradation rate of 1 h^{-1} in the WWTP, elimination in a WWTP by biological degradation, adsorption and volatilisation can be estimated with the model SimpleTreat 3.0. Due to this calculation model the elimination in the WWTP is 87.4 % (mainly biodegradation); 87.3 % are covered by biological degradation and 12.6 % are discharged to surface waters. Volatilisation into the atmosphere and adsorption to sludge are negligible.

The estimation result is presented in table 3.4:

Table 3.4) Distribution in WWTP

Compartment	% 2-ethoxyethanol
to air	0.0
to water	12.6
to sludge	< 0.1
degraded	87.3
total removal	87.4

Accumulation

Tests on bioaccumulation are not available for 2-ethoxyethanol. The measured log Pow of – 0.43 does not provide any indication of a relevant bioaccumulation potential.

The calculated K_{oc} value of 6.3 l/kg (see Appendix A1 for the calculation) also does not indicate a significant geoaccumulation potential.

3.1.2 Aquatic compartment (incl. sediment)

Releases into the waste water might occur during production, use as an intermediate and use as solvent. The exposure data submitted by the remaining company (INEOS 2006) are used to predict environmental concentrations of 2-ethoxyethanol in surface water.

The exposure scenario is based on the A- and B-tables of the TGD, App. I.

3.1.2.1 Determination of the $Clocal_{water}$ during production and processing at one site with specific waste water treatment plant (site-specific approach)

Using the currently available information submitted by the manufacturer, specific exposure calculation can be performed for site A. According to this information, releases can only be expected 5 days per year. During the last years, production volumes did not follow a clear trend (see 2.1). Hence, as a realistic worst case, the production volume assumed for the estimation is 1000 t/a (average production volume over the years 2000 to 2005).

Table 3.5) Production and processing at Site A

Types of use	Production and processing at Site A
Tonnage (t/a)	1000
Main category	non-dispersive use (Ic)
Industrial category	3
Life cycle step	production and processing
Number of days	5 (specific data)
Emission factor to waste water	0.375% (based on specific data)
Fraction of emission directed to water	12.6% (based on SimpleTreat)
total emission to waste water (t/a)	3.75
Size of STP (m^3/d)	6480
River flow rate	5 m^3/s
Dilution factor	68
$Clocal_{effl.}$ (mg/l)	14,6
$Clocal_{water}$ (µg/l)	215.52

Using this assumptions, a $Clocal_{water}$ of **215.5 µg/l** is predicted for the remaining producer of 2-ethoxyethanol in the EU (see App. A2)

3.1.2.2 Determination of the $Clocal_{water}$ during processing into solvents and intermediates (generic approach)

According to the leading company 2-ethoxyethanol is used as solvent and intermediate only for industrial applications in chemical industries (IC 003). The quantitative distribution of the application areas described in chapter 2.2 is used for the exposure assessment.

The results of the calculations of the $Clocal_{water}$ are summarised in the following table.

Table 3.6) $Clocal_{water}$ during processing into solvents and intermediates

Types of use	solvent in chemical industry	Intermediate in chemical industry
Tonnage (t/a)	200	800
Main category	non-dispersive use (Ic)	non-dispersive use (Ic)
Industrial category Use category	3 48 (solvent)	3 33 (intermediate)
Life cycle step	processing	processing
Number of days	40 (B-table 3.2)	80 (B-table 3.2)
Emission factor to waste water	0.02 (A-table 3.3)	0.02 (A-table3.3)
Fraction of emission directed to waste water	0.5 (B-table 3.2)	0.4 (B-table 3.2)
total emission to waste water (t/a)	4	16
Size of STP (m^3/d)	10000	10000
Dilution factor	40	40
$Clocal_{effl.}$ (mg/l)	0,63	1.01
$Clocal_{water}$ (µg/l)	15,75	25.23

See appendix A2 for the calculation

The generic approach for the $Clocal_{water}$ gives a concentration of 15.8 µg/l for the use as solvent and 25.2 µg/l for the use as intermediate.

3.1.2.3 Data on occurrence in the hydrosphere

No measured values related to the occurrence of 2-ethoxyethanol in the hydrosphere are available.

3.1.2.4 Sediment

Data on the occurrence in sediment do not exist for 2-ethoxyethanol. According to the known physico-chemical properties, there is no indication that the chemical is distributed into sediment in a relevant amount. Considering this, and the physico-chemical properties of the substance, accumulation in sediment can be excluded.

3.1.3 Atmosphere

In the case of the production of 2-ethoxyethanol in the EU (site A), the complete release into the atmosphere is estimated to be 0.015 t/a. No further information is available with regard to the release into the atmosphere during the processing, formulation and use of the substance.

Therefore, the releases into the atmosphere are calculated in accordance with the TGD (A- and B-tables in chapter 3 appendix I).

Using the SIMPLETREAT model, with regard to 2-ethoxyethanol, release from industrial waste water treatment plants as a result of evaporation into the air is estimated as approx. 0.1 % of the quantity of the substance entering the waste water treatment plant.

Based on the site specific, measured exposure data of the one production site for 2-ethoxyethanol a resulting air concentration of **0.06 µg/m^3** and an annual deposition quantity of **0.01 µg/m^2 d** is calculated for this site.

Taking into consideration the current processing and use volumes and the exposure tables in chapter 3, appendix I of the TGD and the SIMPLETREAT model, it is possible to calculate the releases into the atmosphere and the resulting deposition quantities according to the physico-chemical properties of the substance and the quantities used (see table 3.4). The results of the calculations are summarised in the following table 3.7:

Table 3.7) Clocal$_{air}$ during processing into solvents and intermediates

Types of use	**solvent in chemical industry**	**Intermediate in chemical industry**
Tonnage (t/a)	200	800
Main category	non-dispersive use (Ic)	non-dispersive use (Ic)
Industrial category Use category	3 48 (solvent)	3 33 (intermediate)
Life cycle step	processing	processing
Number of days	40 (B-table 3.2)	80 (B-table 3.2)
Release factor to air	0.01 (A-table 3.3)	0.01 (A-table3.3)
Fraction of main source	0.5 (B-table 3.2)	0.4 (B-table 3.2)
Direct emission to air (t/a)	2.0	8.0
Annual deposition (µg/m^2 d)	1.1	3.5
Clocal$_{air}$ (µg/m^3)	6.9	11

See appendix A3 for the calculation

3.1.4 Terrestrial compartment

No measured data of 2-ethoxyethanol in the terrestrial environment are available. Based on the SIMPLETREAT model (see chapter 3.1.1) the adsorption of 2-ethoxyethanol to sewage sludge is negligible. Hence, the release to soil with sewage sludge application in agriculture is not considered in the risk assessment.

Consequently, 2-ethoxyethanol might be released to soil only as a result of deposition from the atmosphere. In this regard, the point sources in the use of the substance as an intermediate in industrial industries might contribute to a certain degree to air releases (see chapter 3.1.3).

The release of the substances to the soil according to these scenarios is given in the following table.

Table 3.8) $PEClocal_{soil\text{-}porewater}$ and $Clocal_{soil}$ for 2-ethoxyethanol

Scenario	**route of exposure**	**$PEClocal_{soil\text{-}porew}$ in µg/l**	**$Clocal_{soil}$ in µg/kg**
use as intermediate	deposition	1.5	0.34

(See appendix A4 for calculation)

3.1.5 Non compartment specific exposure relevant to the food chain (secondary poisoning)

Since there is no indication of 2-ethoxyethanol possessing a bioaccumulation potential, a risk characterisation for exposure via the food chain is not necessary.

3.1.6 Other non industrial emissions

Due to the remaining uses, non-industrial emissions of 2-ethoxyethanol can be excluded (see chapter 2.2).

3.1.2 Regional exposure consideration

The only production site with an assumed production of 1000 t/a 2-ethoxyethanol is located in the considered region.

To determine regional/continental background concentrations, all releases, from point and diffuse sources of the formulation, processing and use of 2-ethoxyethanol are considered. 100% of the total exposure quantity from production and processing is taken into account for the continental model and for the defined regional EU standard model (densely populated area of 200 **x** 200 km with 20 million inhabitants). This conservative assumption is used for the first approximation of the regional exposure assessment.

No direct release into the soil was identified. Diffuse release only might occur as a result of dispersal processes. Release might therefore be expected by deposition from the air (see chapter 3.1.4).

Regarding industrial plants it can be assumed that equipment to extract solvent from the exhaust air is installed, at least to a certain degree. Therefore, the calculated releases into the atmosphere in the following tables represent the worst case assumption with no recuperation of solvent from exhaust air, using the A- and B – tables of TGD.

Since there is no remaining relevant private use of 2-ethoxyethanol in EU (INEOS 2006), for all releases into the hydrosphere a scenario is assumed, in which 87.4 % of the substance is biodegraded in industrial waste water treatment plants.

The individual environmental releases for 2-ethoxyethanol are summarised in table 3.9.

Table 3.9) Environmental releases

field of application	**ratio reg./cont.**	**release to WWTPs (t/a)**	**release into the atmosphere (t/a)**
production	100/0	5.75	0.01
use as intermediate in chemical industry	100/0	16.0	8.0
use as solvent in chemical industry	100/0	4.0	2.0
Total		**25.75**	**10.01**

In the calculation for the continental and regional model the individual releases are as follows:

Table 3.10) Continental and regional releases

	continental model (kg/d)	**regional model (kg/d)**
to air	0.301	0
to soil	0	0
to hydrosphere (direct)	0	0
to WWTPs	65.1	0

The regional PECs resulting from the EUSES 2.0.3 calculations are given in table 3.11.

Table 3.11) PEC regional for water, soil and air

	$PEC_{regional_{water}}$	**$PEC_{regional_{soil}}$**	**$PEC_{regional_{air}}$**
2-ethoxy-ethanol	0.03 µg/l	$3 \cdot 10^{-4}$ $\mu g/kg_{wwt}$	$5.6 \cdot 10^{-6}$ mg/m^3

Further details are presented in appendix A5.

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

3.2.1 Aquatic compartment (incl. sediment)

The dataset concerning acute effects on aquatic organisms is complete. However, no long-term studies using fish are available. For the determination of effects to the aquatic compartment four tests with fish, six tests with aquatic invertebrates and two algae test were assessed.

The 2-ethoxyethanol short-term toxicity studies with fish are summarised in table 3.12.

3.12) Short-term toxicity studies for 2-ethoxyethanol

Test organism	LC_{50}(48h) [g/l]	LC_{50}(24h) [g/l]	LC_{50}(96h) [g/l]	Test - method	Reference	Reliability
Carassius auratus		> 5.0		APHA 1971, No. 231	Bridié et al., 1979a	1
Lepomis macrochirus	n.s.	n.s.	> 10	n.s.	Dawson et al., 1975,1977	2
Leuciscus idus	> 10	n.s.		DIN 38412	Hüls AG, 1982	1
Menidia beryllina	n.s.	n.s.	> 10	n.s.	Dawson et al., 1975,1977	2

n.s. = not specified

In an additional further toxicity test using 2-3 month-old guppies (*Poecilia reticulata*) Könemann (1981) determined a LC_{50} value of 16.3 g ethyl glycol/l (not specified whether nominal or effective concentrations) after 7-day exposure under semi-static test conditions (daily renewal of the test solution; oxygen content > 5 mg/l; 22±1 °C, water hardness: 25 mg $CaCO_3$/l; pH value not given, reliability 2).

The following table 3.13 shows the 2-ethoxyethanol toxicity studies on aquatic invertebrates.

Table 3.13) Toxicity studies on aquatic invertebrates

Test organism	Duration	Result [g/l]	Test method	Reference	Reliability
Daphnia magna	24 h	EC_{50} > 10	DIN 38412 part 11	Hüls AG, 1987b	2
Daphnia magna	48 h	IC_{50} = 7.7 motility	static	Hermens et al., 1984	2
Artemia salina	24 h	NOEC =10 mortality	static	Price et al., 1974	2
Hydra attenuata/ adults	92 h	MEC* = 28	partly unspecified	Johnson et al.1984	2
Hydra attenuata/embryos	92 h	MEC* = 5.6	partly unspecified	Johnson et al.1984	2
Daphnia magna	21 d	NOEC > 0.1 reproduction	UBA GL, 1984	Hüls AG, 1988b	2

*MEC = minimum toxic effect concentration

Table 3.14 shows the toxicity of 2-ethoxyethanol to the alga *Scenedesmus subspicatus*. The results are given as nominal concentrations.

Table 3.14) Toxicity of 2-ethoxyethanol to the alga *Scenedesmus subspicatus*

Criterion	Duration [h]	Result [g/l]	Test procedure	Reference	Reliability
Assimilation inhibition	24	EC_0> 10	DIN 38412, L 12	Hüls AG, 1987a	2
Growth inhibition	72	EC_0 > 1.0	UBA-GL, 1984	Hüls AG 1988a	2

$PNEC_{water}$-calculations

With the exception of the (nominal) 48 h EC_{50} for *Daphnia magna*, no effects were observed up to the highest concentrations tested, and no. precise effect value was determined in the ecotoxicological tests using fish, invertebrates and algae.

The various results obtained from tests in which no effects were observed within the concentration range tested are indicative of the very low toxicity of 2-ethoxyethanol.This is also true for the long-term result reported for *Daphnia* (NOEC, 21 d, *D. magna*: ≥ 0.1 g/l, Hüls AG, 1988b).

As supplementary result an indicative value of 5.6 g/l determined as MEC (minimum toxic effect concentration, effect rate not quantified) in a 96 h test, of Johnson et al. (1984) using artificial "embryos" of *Hydra attenuata* was determined. In view of the test design that MEC might be compared to a LOEC with an effect rate below 50 %. Hence a higher short-term sensitivity of *Hydra* compared to that of *Daphnia* is unlikely.

As a worst-case scenario the NOEC for Daphnia magna (≥ 100 mg/l, Hüls AG 1988) is used for the PNEC derivation. Since information about chronic effects is available for two species from different trophic levels (algae, invertebrates), according to the TGD an assessment factor of 50 is applied, leading to a

$PNEC_{water}$ ≥ 2.0 mg/l

$PNEC_{sediment}$ calculations

For 2-ethoxyethanol, no information on sediment tests with benthic organisms is available. Furthermore, there is no relevant adsorption to sediment. Therefore, the derivation of a $PNEC_{sediment}$ value is not necessary.

$PNEC_{microorganisms}$ calculations

There are several tests concerning the toxicity of 2-ethoxyethanol to bacteria.

In a growth inhibition test according to Bringmann and Kühn (Hüls, 1995c; test conditions according to the standardised DIN 38412, part 8) an EC_{10} of 1.7 g/l was determined. The test organism *Pseudomonas putida* was incubated in the presence of different concentrations during 18 hours at 25 °C. The inhibition was determined by turbidity measurements.

Kupferle (1991) carried out a test on respiratory inhibition of non adapted activated sewage. 2-ethoxyethanol concentrations up to 10 g/l were not toxic.

According to an DIN 38412 draft (part 12 was stated as a reference, but does not exist) an EC_0 of > 10 g/l was determined following 24 hour exposure with a mixed bacterial culture (Hüls, 1987c). As the documentation is insufficient, this result cannot be considered for PNEC derivations.

The microbial toxicity of 2-ethoxyethanol was also assessed using bacteria isolated from industrial wastewater (not further specified). The test substance was added to the agar and incubated for 48 hours at 35 ° C. The formed colonies were counted. An EC_{10} of 6.9 g/l and an EC50 of 17 g/l was determined (Cho et al., 1989).

In addition, during an examination of the properties of anti-icing additives for aircraft fuels, the toxic effects of 2-ethoxyethanol for two fungi (*Cladosporium resinae*, *Gliomastix* sp.), yeast (*Candida* sp.) and the bacterium *Pseudomonas aeruginosa* a 2-phase test medium (50 % fuel of aircraft and 50 % water) was tested using different 2-ethoxyethanol concentrations by incubating the test organisms for 4 months. Initial toxic effects were observed at concentrations ranging from 2 - 17 %. Under anaerobic conditions with sulphate reducing bacteria, toxic effects started at 5 - 10 % (60 % fuel and 60 % water; 3 month incubation) (Neihof and Bailey, 1987).

Conclusions

The available data indicate that 2-ethoxyethanol is not toxic to micro-organisms. Nevertheless, a tentative $PNEC_{STP}$ may be derived based on these data. The lowest EC_{10} determined in a standard test was 1.73 g/l. According to the TGD, the $PNEC_{STP}$ is set equal to a $NOEC/EC_{10}$ from a test performed with 'specific' bacterial populations like *Pseudomonas putida*. The following PNEC estimation can be performed:

$PNEC_{STP} = EC_{10}$ = 1.73 g/l

3.2.2 Atmosphere

No ecotoxicological data are available for this environmental compartment.

3.2.3 Terrestrial compartment

No tests on terrestrial organisms are available. The only information regarding the terrestrial compartment is a test with a wild-type strain of *Drosophila melanogaster* (fruit fly):

Adults of both sexes were exposed by Schuler et al. (1985) to different ethyl glycol concentrations contained in a mixture of 2.5 g Drosophila medium and 7.5 ml of distilled water. The exposure continued from the deposited eggs through three instar stages to pupa formation. Compared to unexposed flies, the adults hatched showed distinct morphological aberrations (shortened or bent bristles as well as wing defects), increasing in their incidence with 2-ethoxyethanol concentration (LOELs: 6.5 – 15.8 mg/l).

$PNEC_{soil}$ calculations

Since *Drosophila* is not representative for soil fauna and, in addition, the effect data reported in view of the applied substrate are difficult to interpret, the test results are not suitable for the PNEC derivation.

Consequently, for both ethyl glycol and ethyl glycol acetate only a screening approach based on the equilibrium partitioning method (TGD, subchapter 3.6.2.1, equation 56) is feasible:

$$PNEC_{soil} = \frac{Ksoil\text{-}water \cdot PNEC_{water} \cdot 1000}{Rho_{soil}}$$

$$\textbf{PNEC}_{\textbf{soil}} \textbf{ mg/kg (wwt)} = \frac{0.388 \times 2.0 \text{ mg/L} \times 1000}{1.73 \text{ kg/L}} = \geq \textbf{456}$$

3.2 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

Waste water treatment plants

The highest discharge concentration for waste water treatment plants was calculated as **14.6 mg/l** for the production and processing at the only production site. The concentration for the use as solvent in chemical industry is **0.63 mg/l** and for the use as intermediate **1.01 mg/l**. Generic models are used for the calculation of the $Clocal_{effl.}$ No specific information is available for this area of use of the substance or for the exposure in the environment. Consequently, standard scenarios had to be used for the calculation of the concentrations in the waste water treatment plants (see 3.1.2.1 and 3.1.2.2).

Taking into consideration a **$PNEC_{microorganisms}$** of **1730 mg/l**, the $Clocal_{effl.}$/PNEC ratios are given in the table 3.15.

Table 3.15) $Clocal_{effl.}$/PNEC ratios for WWTP

Field of application	ratio
Production	0,008
Use as intermediate	0,0006
Use as solvent	0,0004

Since the $Clocal_{effl.}$/PNEC ratio is **< 1**, no risk is expected for microbial populations in the WWTP.

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.

Aquatic environments

The PEC/PNEC ratios for production, processing and use are summarised in the following table.

The calculation is based on a **PNEC** of **≥ 2000 µg/l** and **$Clocal_{water}$** values from tables in chapter 3.1.2.

$PECregional_{water}$ = 0.03 µg/l

Table 3.16) PEC/PNEC ratios for the aquatic compartment

Company/area of use	$Clocal_{water}$+ $PEC_{regional}$ = PEC_{local} in µg/l	PEC/PNECwater
Production at site A	215.5 + 0.03 = 215.53	0.107
Solvent in chemical industry	15.8 + 0.03 = 15.83	0.008
Intermediate in chemical industry	25.2 + 0.03 = 25.23	0.013

Based on the conservative approaches for the exposure assessment all PEC/PNEC ratios are far below 1. Hence, no risk for aquatic organisms is expected due to production, processing and use of 2-ethoxyethanol.

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.

Sediment

No data on the occurrence in sediment or investigations of the effect on benthic organisms are available in connection with 2-ethoxyethanol. According to the available physico-chemical properties of the substance, relevant distribution into the sediment or accumulation in sediment can be excluded. There is no need for a separate risk consideration for this compartment.

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.

3.3.2 Atmosphere

Due to the atmospheric half-life of 2-ethoxyethanol ($t_{1/2}$ = approx. 25h), abiotic effects on the atmosphere, such as global warming and ozone depletion, are not to be expected.

During production and processing there is a monitored average mass loss via the vents of the distillation columns of approximately 0.01 kg/t leading to a local air concentration of $5.6 \cdot 10^{-4}$ mg/m^3.For the industrial use of 2-ethoxyethanol as intermediate the highest calculated air concentration is approximately 11 µg/m^3.

Since no data are available on the ecotoxicological effect of the substance in connection with this environmental compartment, it is not possible to undertake a quantitative assessment of this environmental compartment. On the basis of the available information on the substance, the performance of tests is not considered necessary.

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.

3.3.3 Terrestrial compartment

Relevant releases into the terrestrial compartment might only occur from atmospherical deposition. The highest deposition rate is predicted following industrial use as an intermediate. Taking this into consideration, concentrations for 2-ethoxyethanol amounting to a $PEClocal_{soil}$ of **0.34 µg/kg** (dw) in soil and **1.5 µg/l** in soil pore water were estimated.

Table 3.17) PEC/PNEC ratio for the terrestrial compartment

Scenario	**$PEClocal_{soil}$ in µg/kg**	**PEC/PNEC**	**$PEClocal_{soil\text{-}porewater}$ in µg/l**	**PEC/PNEC**
2-ethoxyethanol use as intermediate	0.34	< 0.001	1.5	< 0.001

The PEC/PNEC ratio for soil is based on the $PEClocal_{soil}$ and the PNECsoil (≥ 456,000 µg/kg) derived using equilibrium partitioning. Based on these values a **PEC/PNEC** ratio of **$3.3 \cdot 10^{-6}$** for **2-ethoxyethanol** is calculated. Furthermore, the PEClocal soil-porewater is compared with the aquatic PNEC (> 2000 µg/l) resulting in a PEC/PNEC ratio of < 0.01. There is no indication of a risk to the terrestrial environmental compartment.

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.

3.3.4 PBT-assessment

The following table shows the criteria as defined in the TGD to identify PBT/vPvB substances, and the values relevant for 2-ethoxyethanol.

Table 3.18) Data for 2-ethoxyethanol and PBT/vPvB criteria according to TGD

Criterion	PBT-criteria	vPvB-criteria	2-ethoxyethanol
P	Half-life > 60 d in marine water or > 40 d in freshwater or half-life > 180 d in marine sediment or > 120 d in freshwater sediment	Half-life > 60 d in marine- or freshwater or half-life > 180 d in marine or freshwater sediment	readily biodegradable
B	BCF > 2000	BCF > 5000	BCF (fish): no data
T	Chronic NOEC < 0.01 mg/l or CMR or endocrine disrupting effects	Not applicable	all ecotoxicological results > 0.01 mg/l

2-ethoxyethanol has to be considered as readily biodegradable therefore it does not fulfil the P-criterion. Due to the Pow of –0.43 it is considered as unlikely that the substance accumulates in aquatic organisms. BCF-values are not known. All toxicological studies (chronic and acute studies, see chapter 3.2.1) results in values > 0.01 mg/l.

It can be concluded that 2-ethoxyethanol does not meet the PBT or vPvB criteria.

According to the information from industry no sites producing or processing 2-ethoxyethanol are located near the sea. Therefore a marine risk assessment was not conducted.

3.3.5 Non compartment specific effects relevant to the food chain (secondary poisoning)

Since there is no indication that 2-ethoxyethanol exhibits a bioaccumulation potential, a risk characterisation for exposure via the food chain is considered not necessary.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

According to information from the industry there is only one production site of 2-ethoxyethanol in the EU, where currently approx. 100 t/year are produced. There is no known import from outside of the EU.

Based on the latest information (INEOS 2006), there is no remaining wide dispersive use of 2-ethoxyethanol outside the chemical industry. The current use pattern is as follows:

- processed to intermediates in the chemical industry: 80%
- solvent use in the chemical industry: 20 %

In the chemical industry 2-ethoxyethanol is processed to intermediates such as 2-ethoxyethanol tert.butyl ether.

On account of the risk of adverse development effects 2-ethoxyethanol is replaces by other substances in Germany during the last years. In 1999 producers started a voluntary programme to control the application and use of 2-ethoxyethanol. Products shall not be sold for use in:

- consumer goods / household products
- cosmetics
- pesticide formulations
- pharmaceutical preparations and medicines
- photo-resist mixtures for semi-conductor fabrication
- applications where exposure is poorly controlled.

Furthermore, the European Technical Committee “printing inks“ excluded 2-ethoxyethanol from the production and distribution of printing inks. According to information from industry, 2-ethoxyethanol is no longer used in printing inks or in the manufacturing of electronic components.

In the EU, the production quantity has declined during the last 5 years (2002 – 2006) from 1360 t to 100 t (520 t in 2005 to 100 t in 2006). However, it has to be kept in mind, that 2-ethoxyethanol might current on the market as a component of different products. Since the reduction of the production volume is quite high, it is assumed, that within the coming years no 2-ethoxyethanol will be on the market. Therefore, this RAR consider only the production of 2-ethoxyethanol and its use as a chemical intermediate.

For workers the inhalation and dermal routes of exposure are likely to occur.

4.1.1.2 Occupational exposure

Industrial activities using 2-ethoxyethanol present opportunities for occupational exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

The following occupational exposure limits (OEL) and short term exposure levels (STEL) apply in the EU (Ariel, 2006):

Country	OEL (mg/m^3)	STEL (mg/m^3)
Greece (2001)	74	-
United Kingdom (2005)	37	-
France (2005)	19	-
Germany (2006), Switzerland (2005)	19	152
Austria (2006)	19	76
Sweden (2005)	19	40
The Netherlands (2006)	19	38
Denmark (2005), Iceland (2001)	18.5	-
Belgium (2002), Norway (2003), Ireland (2002), Portugal (2004), Spain (2006), Italy (2006), USA (ACGIH) (2006)	18	-
Finland (2005)	7.5	-

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 95^{th} percentile are derived as representing reasonable worst case situations. For the purpose of exposure assessment only measurement data later than 1990, if available, are taken.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

There is an agreement between the memberstates, within the framework of existing substances, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of forearms and hands by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered, if information is provided, that for a certain scenario gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, test according to DIN EN 374 are taken as a criteria. For most down stream uses it is commonly known, that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since no quantitative information on dermal exposure is available, the EASE model is used for assessing dermal exposure.

The following scenario is regarded to be relevant for occupational exposure:

Production of 2-ethoxyethanol and further processing as an intermediate (4.1.1.2.1)

4.1.1.2.1 Production and further processing of 2-ethoxyethanol in the large-scale chemical industry (scenario 1)

2-Ethoxyethanol is the reaction product of ethanol and ethylene oxide in the presence of a base catalyst. The catalyst is made up in a pre batch with ethanol and fed in the ethanol stream to the pipe reactor. Subsequently ethylene oxide is added in a static mixer. After preheating the mixture up to reaction temperature it flows to the pipe reactor where the reaction proceeds in a range of 150-200 °C and 15 bar. The reaction takes place in an excess amount of ethanol to control exotherm & selectivity towards the endproduct. The product stream from the 2-ethoxyethanol tank reactor is fed to an alcohol removal column where the excess ethanol is removed from the crude gylcolether mixture. The ethanol is recycled back to the pipe reactor. In order to recover the individual glycolether products at high purity level the bottom productstream flows to the purification section which consists of 2 columns called the cellostill and the carbistill. In the cellostill the topstream is the highly purified 2-ethoxyethanol. The bottom stream of the last vacuum column contains the heavier glycolethers. All these glycolether products are cooled and routed to the storage where they are stored under a nitrogen blanket (INEOS, 2006). It is to be assumed that the manufacturing process take place continuously in closed systems.

The production is performed in one campaign per year lasting 10-15 days. 2-Ethoxyethanol is transferred into tanker (5 tankers/campaign, duration 1 hour/tanker) or is drummed (500 drums/campaign, 120 drums/hour). According to information provided by industry approx. 36 workers (3 potentially exposed per 8 hour-shift) are employed in the production of 2-ethoxyethanol (INEOS, 2006).

It is to be assumed, that in-company transfer occurs via pipeline systems and by means of pumping. Exposure associated with transporting the chemical would result from loading, unloading, coupling and uncoupling transfer lines and drumming operations.

For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached, e.g. during filling, cleaning, maintenance, repair works and taking of samples. Inhalation exposure in other fields is normally minimised by technical equipment (e.g. specially designed filling stations, local exhaust ventilation (LEV)).

Workers normally use personal protection equipment (PPE, here: gloves, eye glasses) and, during cleaning activities, respiratory protection in addition.

For the uses of 2-ethoxyethanol as a industrial solvent or uses in chemical laboratories, it is assumed that these applications are performed in closed systems and under control measures.

Inhalation exposure

Measured data

The producer (only since 1998) submitted workplace monitoring data for the production of 2-ethoxyethanol.

From 1998 to 2006 exposure levels to 2-ethoxyethanol were < 0.01 mg/m³ to 5.3 mg/m³ (TWA, 96 samples), with 95th percentile of 3.0 mg/m³ (TWA) and a median of < 0.01 mg/m³ (TWA). The measurements were taken mainly at the production process, and only a few during loading of a tanker and drumming of product.

Based on the measurement results an 8 h TWA of 3.0 mg/m³ is regarded as representing a reasonable worst case situation.

Modelled data

EASE estimation (EASE for Windows Version 2.0, Aug. 1997)

Exposure by inhalation to vapour during the production and further use (chemical intermediate, industrial solvent) with local exhaust ventilation (vapour pressure: 530 Pa).

Input parameters:	T = 20 °C, non dispersive use, LEV present
Level of exposure:	3.8 – 11.4 mg/m³ (1 - 3 ml/ m³)

Because the vapour pressure of 2-ethoxyethanol of 530 Pa is at the lower limit of the EASE model volatility range (500 –1500 Pa), it can be concluded, that the predicted exposure level lies at the lower limit, here 3.8 mg/m^3.

Summary of the exposure level

Inhalation exposure has to be assessed for production and further processing of 2-ethoxyethanol in fields with high levels of protection, e.g. in the large-scale chemical industry.

For the assessment of health risks from inhalation exposure to 2-ethoxyethanol during the production and further processing an 8 h-time weighed average concentration (8 h TWA) of 3 mg/m^3 (95th percentile) should be taken to represent a reasonable worst case situation. This level is in a good agreement with the EASE estimation.

It is to be assumed that the substance is processed in one campaign per year. Consequently, the duration and the frequency of exposure to 2-ethoxyethanol are assumed to be 10-15 days per year and for the entire length of shift.

Dermal exposure

When producing and further processing 2-ethoxyethanol dermal exposure could occur during activities like drumming, loading (tanker), coupling and decoupling of transfer lines, sampling, cleaning, maintenance and repair work. The main source of potential exposure is during drumming activities.

Modelled data

According to the EASE model, potential dermal exposure is assessed as follows:

Input parameters:	Non dispersive use, direct handling, intermittent
Level of exposure:	0.1 – 1 mg/cm^2/day.

Considering an exposed area of 210 cm^2 (this is equivalent to half of one hand) the model yields an exposure level of 21 - 210 mg/person/day. Default assumption of reasonable worst-case dermal exposure for drumming of liquids to be assessed by EASE before taking account of modifying factors (TGD Part 1_2ed, 2003).
For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance. According to information provided by the industry (safety data sheets), in the case of 2-ethoxyethanol, suitable gloves tested according to EN 374 are worn (material: butyl rubber). As a rule, for the use of suitable gloves, low levels of daily dermal exposure are to be expected. However, in spite of this, dermal exposure may occur due to e.g.

- unintended contamination during the handling of used gloves,
- limited protection of suitable gloves at real working conditions (e.g. mechanical stress),
- time of use exceeding the permeation time of the gloves with regard to the substance.

For the use of suitable gloves a protection efficiency of 90 % is considered leading to exposure levels of 2.1 – 21 mg/p/day. The upper level regarded to represent the reasonable worst case.

Summary of the exposure level

For assessing the health risks from daily dermal exposure in the area of production and further processing of 2-ethoxyethanol, an exposure level of 21 mg/person/day should be taken. This exposure assessment is based on the assumption, that gloves are suitable for the protection against 2-ethoxyethanol.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Summary of occupational exposure

Based on the available information, the exposure assessment reveals that handling 2-ethoxyethanol during production and further processing is the main source for occupational exposure.

Other uses (see 4.1.1.1) have declined during the last years.

For occupational exposure there is only one scenario:

Production of 2-ethoxyethanol and further processing as an intermediate

Relevant inhalation and dermal exposure levels are given in table 4.1.1.2.2.

For the large scale chemical industry, it is assumed that the production and further processing of 2-ethoxyethanol is mainly performed in closed systems. Exposure occurs if the systems are breached for certain activities, e.g. drumming.

As concerning dermal exposure producers provided information that suitable gloves (tested according to EN 374) are used regularly. This is considered in assessing dermal exposure during production and further processing using the EASE model assuming a protection efficiency of 90 %.

Table 4.1.1.2.2: Conclusions of the occupational exposure assessment

Scenario	Activity [1]	Frequence Days/year	Duration Hours/day	Inhalation				Dermal			
				Reasonable worst case		Typical concentration		Reasonable worst case		Typical concentration	
				Unit	Method[2]	Unit	Method [2]	Unit	Method [2]	Unit	Method [2]
Production											

Inhalation exposure (RWC)								
Scenario number, Area of production and use	**Form of exposure**	**Activity**	**Duration [h/day]**	**Frequency [days/year]**	**Shift average concentration [mg/m^3]**	**Method**	**Short-term concentration [mg/m^3]**	**Method**
Production and further processing as an intermediate	vapour (liquid)	drumming, loading, cleaning, maintenance	shift length (assumed)	10-15 (one campaign)	3	95th percentile	-	-

Dermal exposure (RWC)								
Scenario number, Area of production and use	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Contact level [1)]**	**Level of exposure [mg/cm^2/day]**	**Exposed area [cm^2]**	**Shift average [mg/person/day]**	**Method (use of gloves)**
Production and further processing as an intermediate	liquid	drumming, loading, cleaning, maintenance	10-15 (one campaign)	intermittent	0.1 - 1	210	21	EASE (90 % protection, suitable gloves)

[1)] Contact level according to the EASE model

4.1.1.3 Consumer exposure

According to regulations, products containing >0.5% of 2-ethoxyethanol have to be labelled as toxic since 1993.

There is no indication that since that time new products containing 2-EE have been placed on the market. It may be possible that exposure to 2-ethoxyethanol may happen with some remained of the above mentioned products. Exposure assessment has not to be performed because consumer products on the basis of 2-ethoxyethanol are not available.

2-Ethoxyethanol has been detected on toy-surfaces, in a measurement programme of the danish EPA. In two toys out of a series of 15 measurements performed by Danish EPA 2 and 17 µg/cm² were found. Hansen & Pedersen calculated an oral uptake of 0,55 resp. 5,4 µg/kg per day taking these data. It remains open whether these findings have a systematic background or were found occasionally.

The occurance of 2-ethoxyethanol in cosmetics has been assumed by Mariani et al (1999), however, they did not provide further data for substantiation.

4.1.1.4 Indirect exposure via the environment

In accordance with the TGD, the indirect exposure of man to 2-ethoxyethanol via the environment, e.g. via food, drinking water and air, must be determined. In the form of a worst-case scenario, the most significant point source (in this case the industrial use as intermediate and solvent) is considered for calculation purposes. This result is then compared with a second calculation which is based on the regional background concentrations (see chapter 3.1.7).

The results of these calculations with the corresponding input values are summarised in Appendix A6. It is necessary to note, however, that the calculation model applied is as yet only provisional. It requires revision as soon as further information is available.

The following input parameters were selected:

Table 4.1: Input parameters

	PEC_{local} annual (greatest point source)	regional background concentrations

Concentration in soil	0.4 µg/kg	1.29 • 10-7 mg/kg
Concentration in the surface water	0.006 mg/l	0.03 • 10-3 mg/l
Concentration in the atmosphere	0.002 mg/m3	5.6 • 10-9 mg/m3
Concentration in the ground water	0.002 mg/l	1.18 • 10-6 mg/l

The resultant daily doses for the substance are as follows:

- DOSEtot = 2.67 • 10-3 mg/kg body weight day (local scenario)
- DOSEtot = 9.38 • 10-7 mg/kg body weight day (regional background concentrations)

The calculated uptake quantities result via the following routes:

Table 4.2: Calculated uptake quantities result

Uptake route	% of total uptake for 2-ethoxyethanol	
	local	regional
drinking water	5.95	98.22
fish	0.03	0.49
plant shoot	74.16	0.51
root	0.29	0.65
meat	< 0.01	< 0.01
milk	0.02	0.01
air	19.55	0.48

Plant shoot is the most significant route of uptake for 2-ethoxyethanol in the local approach. In the regional scale this is drinking water.

4.1.1.5 (Combined exposure)

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

4.1.2.1 Toxico-kinetics, metabolism and distribution

A. Absorption and distribution in tissue

Animal data:

Oral route

Medinsky et al. (1990) have studied the disposition of ^{14}C-2-ethoxyethanol in male F344/N rats, which had access for 24 hr to three different concentrations of 2-ethoxyethanol in drinking water (concentrations ranged from 220 to 1940 ppm, which corresponded to 94±22 - 1216 ± 86 µmol/kg bw (n = 4)). Elimination of radioactivity was monitored for 72 hr. 50 – 70 % of the administered radioactivity was excreted in the urine, whereas 10 – 20 % of the administered radioactivity was exhaled as CO_2. The majority of ^{14}C was excreted in the urine or exhaled as $^{14}CO_2$. Exhalation of the unmetabolized glycol ether was a minor route of elimination. In summary, 60 – 90 % of the administered amount was absorbed (see also Table **X**).

Inhalation route

2-Ethoxyethanol in a concentration of 120 µg /ml was detected in blood after a 2-h whole-body exposure of three female Sprague-Dawley rats to 1690 mg 2-ethoxyethanol/m^3 air (420 ppm). If rats were intraperitoneally administered 920 mg ethanol/kg bw before 2-ethoxyethanol inhalation, the 2-ethoxyethanol measured in the blood increased to a mean of 280 µg/ml (Römer et al., 1985).

Male F344/N rats were exposed nose-only to either 20 mg/m^3 (5 ppm) ^{14}C-2-ethoxyethanol for 5 h 40 min or 185 mg/m^3 (46 ppm) ^{14}C-2-ethoxyethanol for 6 h. Within the dose range studied, the absorption and total metabolism of ethoxyethanol were linearly related to the concentration of ethoxyethanol in the exposure atmospheres. 28 % (at 5 ppm) and 29 % (at 46 ppm) of the inhaled amounts were retained (retained percentagepercentage: amount retained/amount inhaled times 100; retained ethoxaethanol is defined as the amounts of ^{14}C-ethoxyethanol equivalents inhaled by a rat during an exposure that is not exhaled as parent compound). Significant percentages of the retained doses were exhaled during (22%) and after (16%) exposure as $^{14}CO_2$. Forty-six percent of the retained dose (the retained dose was defined as the amount of ^{14}C in urine, feces, exhaled $^{14}CO_2$ and ^{14}C remaining in the carcass) was excreted in the urine collected up to 66 hr after exposure. Approximately 10% of the retained dose was detected in the carcass 66 h after exposure (Kennedy et al. 1993). In summary, 28 – 29 % of the administered amount was absorbed (see also Table **X**).

Dermal route

Three different doses of the ^{14}C-labeled 2-ethoxyethanol (40- 360 mg/kg, area of application was 9.4 cm^2, chemicals were dissolved in acetone) were applied to same-sized areas on the clipped backs of male F344/N rats and non-occluded percutaneous absorption was measured. Approximately 20-27 % of the dermally applied dose of 2-ethoxyethanol was absorbed (absorption was defined as radioactivity equivalents present in the carcass (with exception of the skin at the site of application) or excreta within 72 hr postexposure as a percentage of the applied dose) regardless of the applied dose. The majority of the absorbed dose was excreted in the urine. 44 to 47% the applied dose was trapped as volatile ^{14}C from metabolism cage exhaust. These amounts can be due to exhaled $^{14}CO_2$ after metabolism and uptake and also due to substance which evaporated from the skin or substance that had been exhaled unchanged. Fecal excretion and exhalation as $^{14}CO_2$ represented minor routes of excretion. A small amount of the applied ^{14}C (0.3 to 2%) was still present at the site of application 72 h following dosing (Sabourin et al. 1992). In summary, 20 – 27 % of the applied dose was absorbed (see also Table **X**).

Other routes

Repeated ip. dosing (5 times one injection per hour) with 2-ethoxyethanol (360 mg/kg bw) plus ethanol (460 mg/kg bw) resulted in an almost complete accumulation of 2-ethoxyethanol in the blood. The prolonged retention of 2-ethoxyethanol is due to an inhibition of the degradation of this compound, because of a competition with ethanol concerning alcohol dehydrogenase (Römer et al. 1985).

Human data:

Inhalation route

For determination of the respiratory uptake and elimination of 2-ethoxyethanol after inhalation exposure, 10 male volunteers in groups of 5 persons each were exposed for 4 h (with a break at the end of each hour) at rest by means of a respiratory mask to 10, 20 or 40 mg/m^3 2-ethoxyethanol or to 20 mg/m^3 2-ethoxyethanol during physical activity on a bicycle ergometer at 30 or 60 Watt. The uptake quantity was ascertained by measuring the 2-ethoxyethanol concentration in the expired air and the pulmonary ventilation rate. Under all exposure conditions, steady state levels of retention, atmospheric clearance, and rate of uptake were reached immediately after the start of the exposure. There was no indication, therefore, for a possible saturation of 2-ethoxyethanol under the conditions used. About 64% of the inhaled vapour was retained at rest. The rate of uptake was higher as exposure concentration or pulmonary ventilation rate, or both, increased. Respiratory elimination of unchanged 2-ethoxyethanol accounted for $\leq$4% of the total body uptake. There was no indication for the presence of volatile organic compounds other than 2-ethoxyethanol in the expired air (Groeseneken et al. 1986a).

The same experiment (Groeseneken et al., 1986a) was used to determine urinary excretion of ethoxyacetic acid within 42 hours (i.e. within 4 hours during exposure and 38 hours after exposure). Maximal excretion of ethoxyacetic acid was reached three to four hours after the end of the 4-hour exposure period. Based on the measured results for the elimination via urine, a half-life of 21 to 24 h was calculated. An average of 23% of the absorbed 2-ethoxyethanol was recovered in the urine as 2-ethoxyacetic acid, whereby this fraction was independent of the 2-ethoxyethanol uptake. At every time after the exposure, the 2-ethoxyacetic acid excretion was proportional to the ethoxyethanol dose (Groeseneken et al. 1986b).

After reanalysis of the data obtained from the men exposed under resting conditions (Groeseneken et al., 1986a), a mean elimination half-life of 42.0 ± 4.7 h has been calculated which is 6 times higher compared to rats. The elimination of ethoxyacetic acid was not complete after 48 hours. On average, after 48 hours, 23 % of the inhaled 2-ethoxyethanol was excreted as ethoxyacetic acid in the urine. The total recovery of ethoxyacetic acid was estimated to be 30 – 35 % by extrapolation using an elimination half-live of 42 hours (Groeseneken et al., 1988).

Dermal route

Kezic et al. (1997) investigated the absorption of vaporous and liquid 2-ethoxyethanol in two male and three female human volunteers. Dermal exposure to 2-ethoxyetanol vapour (3700 mg/m^3) on an area of about 1000 cm^2 skin of forearm and hand lasted 45 min. Duration of exposure to liquid 2-ethoxyethanol on an area of 27 cm^2 skin from the forearm was 15 min. Dermal uptake was assessed by measurement of the main urinary metabolite ethoxyacetic acid. Vaporised and liquid 2-ethoxyethanol was readily absorbed through the skin: the mean absorption rate for vaporous 2-ethoxyethanol was 19 ± 6 cm/h and the mean absorption rate for liquid 2-ethoxyethanol was 0.7 ±0.3 $mg/cm^2/h$. From combined inhalation and dermal exposure experiments with 2-ethoxyethanol, which have been performed in the same study, it was calculated that when whole body surface is exposed to vapour, the uptake through the skin is estimated to be 42 % of the total uptake of 2-ethoxyethanol.

Table **X**: overview on the absorbed amounts of substance after oral, dermal or inhalation uptake of 2-ethoxyethanol in laboratory animals and humans.

Route	Species / Strain	Amount absorbed	Remarks	References
Oral	Rat (F344/N)	60 – 90 %		Medinsky et al., 1990
Oral	Rat (Sprague-	76- 80 %		Cheever et al.,

	Dawley)			1884
Oral	Rat (Wistar)	36.8 %	Based on urinary excretion of ethoxyacetic acid	Groeseneken et al., 1988
Oral	Rat (Albino rats)	30 %	Based on the urinary excretion of ethoxyacetic acid and N-ethoxyacetyl glycine	Jönsson et al. 1982
Dermal	Rat (F344/N)	20 – 27 %	Skin at the site of application excluded	Sabourin et al., 1992
Dermal	Human volunteers	42 %	From combined inhalation and dermal exposure experiments with 2-ethoxyethanol, which have been performed in the same study, it was calculated that when whole body surface is exposed to vapour, the uptake through the skin is estimated to be 42 % of the total uptake of 2-ethoxyethanol.	Kezic et al., 19971997
Inhalation	Rat (F344/N)	28 – 29 %		Kennedy et al., 1993
Oral	Rat (Albino rats)	30 %	Based on the urinary excretion of ethoxyacetic acid and N-ethoxyacetyl glycine	Jönsson et al. 1982
Inhalation	Human volunteers	64 %	Pulmonary retention	Groeseneken et al. 1986a
Inhalation	Human volunteers	23 %	Based on urinary excretion of ethoxyacetic acid	Groeseneken et al. 1986b
Inhalation	Human volunteers	30 – 35 %		Groeseneken et al. 1986a

B. Metabolism and elimination

Animal data:

The major metabolites of 2-ethoxyethanol after oral administration or inhalation exposure in rats are 2-ethoxyacetic acid, the glycine conjugate of 2-ethoxyacetic acid (Jönsson et al. 1982; Cheever et al. 1984; Groeseneken et al. 1988) and 2-ethylene glycol (Kennedy et al. 1993). Metabolites after administration in rats via drinking water or dermal exposure were 2-ethoxyacetic acid and ethylene glycol. With increasing exposure concentration, excreted amounts of ethoxyacetic acid and ethylene glycol in the urine increased (Medinsky et al. 1990; Sabourin et al. 1992).

Oral route

The routes of ^{14}C-excretion following the administration of a single oral 230 mg/kg bw dose of 2-ethoxyethanol (ethanol-1,2-^{14}C vs. ethoxy-1-^{14}C) to male rats were investigated. Elimination of the ^{14}C by the urinary route accounted for 76 to 80% of the dose within 96 h. The main pathway of biotransformation is oxidation to the corresponding acid. Minor amounts of ethoxyacetic acid were conjugated with glycine. The major metabolites, 2-ethoxyacetic acid and N-ethoxy acetyl glycine, representing 44% vs 30% of the administered dose, were eliminated in the urine. Minor metabolites in the urine, accounting for only 3 to 5% of the administered ^{14}C, were not identified. Unchanged 2-ethoxyethanol was not detected in the urine. The major difference in the metabolic profiles of the two radiochemicals was in the rate and amount of ^{14}C expired via the lung (ethanol-1,2-^{14}C 11.7% of the dose vs. ethoxy-1-^{14}C only 4.6% of the dose). These results show that the ether linkage of 2-ethoxyethanol was cleaved to the extent of at least 11.7% in the rat. Minor amounts of ^{14}C were excreted in the feces (3 to 4.5% of the dose) or remained in the carcass (2 to 4.6% of the dose) at 96 h after treatment. The biological half-life was 9.9 h for the ethoxy-labelled compound and 12.5 h for the ethanol label (Cheever et al. 1984).

Male rats were given single oral doses of 2-ethoxyethanol. The doses ranges from 0.5 to 100 mg/kg bw. The mean elimination half-life of free as well as conjugated 2-ethoxyacetic acid was 7.2 h for all doses. Ethoxyacetic acid was excreted partly as a glycine conjugate (on average 27%), the extent of conjugation being independent of the dose. The relative amount of ethoxyethanol recovered in urine as ethoxyacetic acid was higher: at a dose of 100 mg/kg bw, the recovery mounted to 36.8% (within 60 h) (Groeseneken et al. 1988).

Two Albino rats were given 47 and 465 mg 2-ethoxyethanol/kg bw respectively, by feeding per os. 2-Ethoxyacetic acid and N-ethoxyacetyl glycine were detected and identified as metabolites. The combined excretion of the two metabolites was a estimated to be 30% of the applied dose, for the high as well as for the low dose (Jönsson et al. 1982).

Rats were given one of three concentrations (220 to 1940 ppm ^{14}C-ethoxyethanol) in their drinking water. Elimination of radioactivity was monitored for 72 h. In the urine, 25 to 40% of the administered radioactivity was eliminated as ethoxyacetic acid and 18% as ethylene glycol. Five to 6% of the metabolites were not identified. Less than 5% of the dose was exhaled as unmetabolized ethoxyethanol and 20% as CO_2. With increasing dose administered, the contributions of both ethylene glycol and carbon dioxide to the total excreted radioactivity decreased, and the contribution of ethoxyacetic acid increased (Medinsky et al. 1990).

Inhalation route

One Albino rat was subjected for 1 hour to an atmosphere containing 37000 mg/m³ 2-ethoxyethanol initially. 2-Ethoxyacetic acid and N-ethoxyacetyl glycine were detected and identified as metabolites (urine was collected for 24 h after this exposure). The two metabolites represented approximately 30 % of the applied dose (Jönsson et al. 1982).

Rats were exposed to either 20 mg/m^3 (5 ppm) ^{14}C-2-ethoxyethanol for 5 h 40 min or 185 mg/m^3 (46 ppm) ^{14}C-2-ethoxyethanol for 6 h. The uptake and metabolism of 2-ethoxyethanol was linear in the concentration range studied. The major urinary metabolite was 2-ethoxyacetic acid (22 to 24% of the absorbed dose), which was excreted either as parent substance or as N-ethoxyacetyl glycine. N-ethoxyacetyl glycine and ethylene glycol were identified as minor metabolites excreted in the urine (both approximately in equivalent amounts with 6 to 8% of total metabolites). Additionally, an unknown minor metabolite (representing approximately 1 to 2% of the retained dose) was also detected (Kennedy et al. 1993).

Dermal route

The majority of dermally absorbed 2-ethoxyethanol (the dermally absorbed dose was 20 – 27 %) within the dose range of 40 to 360 mg/kg was excreted in the urine (64 to 77% of *absorbed and metabolized dose*). Ethoxyacetic acid was a major metabolite (50 to 58% (given as percentage of the sum of total urine metabolites and exhaled $^{14}CO_2$)), 13 to 18% (given as percentage of the sum of total urine metabolites and exhaled $^{14}CO_2$) was excreted as ethylene glycol, 24 to 26% (given as percentage of the sum of total urine metabolites and exhaled $^{14}CO_2$) was not identified. Feces contained 6.5 to 14% of absorbed and metabolized radioactoiivity. Exhalation as $^{14}CO_2$, which represented 3.2 to 5.5% of the absorbed and metabolized dose, was a minor route of excretion. In the carcass, an amount of 12 to 16% of absorbed and metabolized dose was retained (Sabourin et al. 1992). There was no significant effect of the dose on the excretion of metabolites.

Human data:

Experimental exposure - inhalation

During a renewed analysis of the urine samples from human volunteers (Groeseneken et al. 1986b) under modified conditions (compilation of the numerous single samples to 12-hour samples), the authors calculated a mean half-life of 42 h for the 2-ethoxyacetic acid excretion in the urine. This evaluation also resulted in a half-life being independent of the exposure concentration. On the basis of this half-life, the authors estimated that a total of about 30 to 35% of the inhaled 2-ethoxyethanol is in the urine. There were no indications for the formation of 2-ethoxyacetic acid conjugates. In man, the recovery of 2-ethoxyacetic acid was higher than in the rat for equivalent low doses of 2-ethoxyethanol (0.5 and 1 mg/kg), indicating that the metabolic conversion of 2-ethoxyethanol to 2-ethoxyacetic acid seemed to be more important in man that in rat (Groeseneken et al. 1988).

Work place exposure - inhalation

The urinary excretion of 2-ethoxyacetic acid was studied in a group of five women occupationally exposed to complex solvent mixtures containing different ethylene glycol derivatives, esters, alcohols, ketone and trichloroethane. Among the glycol ethers the most

important were 2-ethoxyethanol and ethoxyacetic acid. In the first period, urine samples were taken each day for an entire week (5d). In the second period, urine samples were taken during seven workdays after a 12d production stop. The mean combined exposure concentration was 14 mg/m^3. Based on the observations from the first period, a good linear correlation was found between the average exposure over 5 d and the excretion of 2-ethoxyacetic acid at the end of the week. The represented results seem to indicate that the half-life may be longer than 21 to 24 h (Veulemans et al. 1987).

17 persons from a varnish production plant or the ceramic industry were examined for their excretion of 2-ethoxyacetic acid in urine after occupational exposure to 2-ethoxyethanol or 2-ethoxyethyl acetate. Sampling of urine was performed before and during an exposure-free weekend. On Friday, a median value of 29.8 mg/l was determined for 2-ethoxyacetic acid in urine, on monday morning the median value for 2-ethoxyacetic acid in the urine was 10.7 mg/l. Based on the elimination curves a medium half-life of 57 h was calculated. This value is considerably longer than data from experimental exposure (Söhnlein et al. 1992).

In vitro studies

Dugard et al. (1984) analyzed the in vitro permeability of various glycol ethers through human skin by means of diffusion chambers. The entry of the undiluted substance through pieces of epidermis into tritiated water was investigated. The rate of absorption of 2-ethoxyethyl acetate was comparable to that of the parent alcohol (0.8 mg x cm^{-2} x h^{-1}).

Under similar conditions, Barber et al. (1992) found an uptake rate of 1.4 mg x cm^{-2} x h^{-1} in saline solution.

The 2-ethoxyethanol metabolism was studied in vitro by using human hepatocytes. The hepatocyte suspension was incubated for 4 hours with 1.8, 18 and 180 mg ^{14}C- 2-ethoxyethanol/l and the metabolites were identified. 2-Ethoxyacetic acid and ethylene glycol were the main metabolites identified; the formation of the ethylene glycol decreased depending on the concentration (Abstract; Green et al. 1989).

In vitro, 2-ethoxyethanol was readily oxidized to 2-ethoxyacetic acid by an alcohol dehydrogenase isolated from human liver (post mortem). The following values were established: K_m = 6 x 10^{-4} mol/l (ethanol: 1 x 10^{-3} mol/l, and the maximum reaction rate, V_{max} was 44% of the value measured for ethanol (Blair & Vallee 1966).

In another in vitro experiment, the alcohol dehydrogenase activity in the cytosolic fraction of a human liver homogenate (post mortem) was 3 µmol NADH formed / min x mg protein after a 20-min incubation with 3 mg 2-ethoxyethanol/ml at 37°C (after incubation with ethanol, the activity was 5.8 µmol NADH formed / min x mg protein) (Kassam et al. 1989).

Conclusion:

2-Ethoxyethanol is well absorbed via the respiratory tract, the skin and the gastrointestinal tract. The principle metabolites in the urine are 2-ethoxyacetic acid and ethylene glycol. The glycine conjugate of 2-ethoxyacetic acid also occurs in animals, but not in humans. In animal experiments, 2-ethoxyethanol degradation could be inhibited by ethanol. The main route of excretion is via the urine. Feces and exhaled $^{14}CO_2$ represent minor routes of excretion. The half-life for the excretion of 2-ethoxyacetic acid ranged from 21 h (experimental conditions) to 57 h (work place conditions) for humans, but only 7 to 12.5 h in rats. Respiratory

elimination of unchanged 2-ethoxyethanol for humans is ≤4% of the total body uptake. The extent of absorption after oral exposure is assumed to be 100 % for risk characterisation purposes (worst case). Based on human and animal data, 50 % dermal absorption should be taken for risk characterisation purposes. Based on human data, 64 % absorption via the inhalation route is recommended for risk characterisation purposes in humans. For animals on the other hand, lower inhalation absorption percentages can be assumed (30 %).

4.1.2.2 Acute toxicity

Animal data:

Oral

2-Ethoxyethanol has demonstrated low acute oral toxicity in several studies with rats, mice and guinea pigs revealing LD50 values of 2300-4700 mg/kg body weight. Most of the studies were realized in the years 1939-1956 and thus, do not fulfil current guideline standards:

An oral LD50 of ca. 3070 mg/kg bw (3.3 ml/kg) was detected in a study with rats using "concentrated" substances and 1:1 and 1:3 "dilutions". The test substance was administered by stomach tube in single doses, both sexes were used and approximately equally distributed (no differentiation). The following dosages and mortality ratios are stated for ethoxyethanol: 0/10 at 2.6 ml/kg, 8/10 and 4/10 at 3.0 ml/kg, 3/9 at 3.1 ml/kg, 5/10 and 8/10 at 3.3 ml/kg, 6/9 at 3.4 ml/kg, 10/30 and 7/10 at 3.5 ml/kg, 8/9 at 3.7 ml/kg, 7/10 at 3.75 ml/kg, 15/20 and 9/10 at 4.0 ml/kg (Laug et al. 1939).

In the same study an oral LD50 of ca. 4300 mg/kg bw was found for mice (for "concentrated" solutions of 2-ethoxyethanol LD50 between 4.0 and 5.0 ml/kg, for "diluted" solutions of 2-ethoxyethanol between 5.0 and 5.5 ml/kg), here the following dosages and mortality ratios are stated: "concentrated substance": 0/10 at 3.0 ml/kg, 3/20 at 3.5 ml/kg, 6/10 at 4.0 ml/kg; 4/10 at 4.5 ml/kg, 7/10 at 5.0 ml/kg, 10/10 at 6.0 ml/kg. "Diluted substance": 0/10 at 3.0 ml/kg, 2/10 at 3.5 ml/kg, 3/10 at 4.5 ml/kg, 11/30 at 5.0 ml/kg, 6/10 at 5.5 ml/kg, 8/10 at 6.0 ml/kg (Laug et al. 1939).

The respective study with guinea pigs detected an oral LD50 of ca. 2500 mg/kg bw (2.7 ml/kg): For guinea pigs the following dosages and mortality ratios are stated: 1/10 at 2.5 ml/kg, 9/15 at 2.75 ml/kg, 15/20 at 3.0 ml/kg, 13/18 at 3.5 ml/kg, 10/10 at 4.0 ml/kg. For all species nearly the same symptomatic response and pathology is specified: Immediately after application no symptoms were seen. With moderate doses, death was sometimes delayed for 4-6 days; with large doses, death usually occurred in 24-36 hours. Hematuria was noted in nearly all animals, and after death the bladders remained distended with bloody urine. The kidneys of some animals showed extreme tubular degeneration with almost complete necrosis of nearly all of the cortical tubules. About one third of the Bowman's spaces were distended, there was marked congestion. These extensive kidney changes were not frequent, but mild changes always occurred. Hemorrhagic areas in the stomach and intestines were seen uniformly. Liver damage was very mild as were any injuries noted in other organs (Laug et al. 1939).

In a further study with rats an oral LD50 of 3000 mg/kg bw was detected for ethylene glycol monoethyl ether, "commercial grade": The substance was administered to ten rats per dose as 50% aqueous solution by stomach tube. An oral LD50 of 3000 mg/kg was detected with lower limit 2510 mg/kg and upper limit 3590 mg/kg; the slope of the dose-mortality curve was 6.16. No data on clinical signs and no data on necropsy are mentioned. The same study assessed an oral LD50 for guinea pigs: The substance was administered to ten guinea pigs per dose as 50% aqueous solution by stomach tube. An oral LD50 of 1400 mg/kg was detected with lower limit 1220 mg/kg and upper limit 1600 mg/kg; the slope of the dose-mortality curve was 7.75. No data on clinical signs and no data on necropsy are given (Smyth et al. 1941).

For male rats an oral LD50 of 2300 mg/kg bw was found in a study with 99% pure 2-ethoxyethanol: Groups of 2 male rats each were treated with various amounts of the substance - doses of 250, 500, 1000, 2000, 4000 and 8000 mg/kg bw were administered by gavage. LD50 was determined to be 2300 mg/kg bw. Metabolism and excretion was assessed, but no data on clinical signs and no data on necropsy are submitted (Cheever et al. 1984).

With a substance named „CELLOSOLVE Solvent“ (no data on purity) LD50 values of 5.09 ml/kg (4733 mg/kg) body weight and 2.46 ml/kg (2288 mg/kg) bw were detected for rats in a test using 4 groups of 5 male rats each (no further information on that doses) and 3 groups of 5 female rats each (no further information on that doses). Sluggishness, unsteady gait, slow breathing, piloerection, prostration and emaciation were among the signs of toxicity observed. All deaths occurred at 1-2 days. Findings at necropsy included mottled and red lungs, liquid-filled stomachs, dark red and yellow intestines, and bladders filled with dark red liquid. These conditions were evident in the victims, but no remarkable gross lesions were apparent in the survivors (Union Carbide Corp. 1983, unpublished report).

Inhalation

Acute inhalation toxicity of 2-ethoxyethanol is demonstrated to be low by LC50 values assessed with rats: For male rats an inhalative LC50 of 15.2 mg/l after a 4-hours exposure and an inhalative LC50 of 7.36 mg/l after an 8-hours exposure was found: Three out of 6 male rats died after inhalation exposure to 4000 ppm "Cellosolve" (15.2 mg/l) for 4 hours (Carpenter et al. 1956). A study with rats revealed for "Cellosolve" after a single 8-hours inhalation exposure a LC50 value of 7.36 mg/l: The liquid substance was delivered by a motor-driven syringe into a heated evaporator through which an appropriate amount of air was metered. The resultant vapour was then conducted into a desiccator which served as the inhalation chamber for 6 rats. For Cellosolve, a LC50 of 7.36 mg/l (4.01-13.5 mg/l) for a single 8-hours inhalation was detected (Pozzani et al. 1959).

Acute inhalation toxicity of 98% pure 2-ethoxyethanol was assessed within the framework of a study on the reproducibility of the "inhalation-risk test", an OECD method. A maximum non-lethal exposure period (14 days observation post application) for rats was determined in 6 different laboratories after inhalative exposure to ethyl glycol in a nominal concentration of 18-20 mg/l. This concentration was survived by 10/10 rats when exposed for 3 hours: Five female and 5 male rats per group were exposed for 3, 10, 30 minutes and 1, 3, and 7 hours to

saturated vapours of the substance (saturated in air under test conditions at 20º C, nominal concentration 18-21 mg/l) (Klimisch et al. 1988).

In an inhalation hazard test with Ethyl Oxitol (listed as “ethylene glycol monoethyl ether” in the original report, no data on purity) using rats 0/6 rats died after 20.9 mg/l/3 hours (5507 ppm/3 hours) and 6/6 rats died after 20.9 mg/l/7 hours (5507 ppm/7 hours): Dry, oil free laboratory compressed air was conducted through a glass flask at 10 l/min by means of a glass fritt, above which about 120 cm^3 of the test liquid was situated. The portion of the flask containing the test liquid was immersed in a water bath maintained at 20º C. The resulting air/test substance mixture was conducted to the inhalation chamber. Concentrations during exposures were estimated from the weight loss of material from the reservoir, the air flow rate through the generator and the duration of exposure. The flow from the generator was split to supply 2 cylindrical glass tubes each holding 3 animals in line separated from each other by wire mash screens. Total volume of the system was approximately 10 l. Maximum exposure was for 7 hours. If deaths occurred during either the exposure period or the observation period, exposures were repeated for shorter intervals until no deaths occurred in either exposure or observation periods. The saturated concentration at 20º C was detected as 5507 ppm. Test results: After 3 hours of exposure, all 3 male and 3 female rats survived, demonstrating champing during exposure and blood in urine and lethargy post exposure; they all had recovered at day 2. After 7 hours of exposure, all 3 male and 3 female rats died within 24 hours (Shell Research Ltd. 1982, unpublished report).

In a test using CELLOSOLVE Solvent (no data on purity), exposure to dynamically generated substantially saturated substance vapour for a 6-hour period resulted in no deaths among 5 male and 5 female rats. The vapour was produced by passing air (at 2.5 litres/min) through the sample and then through a 9-liter animal chamber (dynamic conditions). No signs of toxicity were noted and necropsy revealed no remarkable gross lesions (Union Carbide Corp. 1983, unpublished report).

The reproductive effects after a single 3-hours inhalation of 2-ethoxyethanol (17 mg/l, ca. 4500 ppm) were assessed in male rats: Saturated substance vapour was generated by blowing air through the test material contained in a glass bubblier. The undiluted vapour was led for 3 hours into 1l glass exposure chambers, each containing 5 male rats housed individually. The rats were observed during exposure and throughout a subsequent 14-day observation period. On day 15 they were killed. 2-Ethoxyethanol caused hematuria and a 20% reduction in testes weight (Doe 1984).

An inhalative LC50 value of 6.4-6.7 mg/l/7 hours was detected in mice for ethylene glycol monoethyl ether with "relative high degree of purity": Relatively high and constant saturation concentrations of the substance in air were obtained by means of a specific apparatus. Even distribution of the substance concentration within the exposure chamber was obtained, maximal concentrations were built up in 45 minutes or less. White mice were exposed, in groups of 14-16 for a 7-hour period: After exposure to 22.0 and 20.3 mg/l all mice died within the 7-hours exposure period; after exposure to 6.4-6.7 mg/l ca. 50% of the mice died within 2 weeks; after exposure to 4.15 mg/l 12.5% of the mice died within one week. Clinical signs: Exposure to vapours was followed by no evidence of typical narcotic action in mice. Following the use of lethal concentrations, some animals were unable to move, and a few appeared analgesic. These effects, however, were associated with marked dyspnea and weakness. With nearly all concentrations the large part of the mortality occurred between 7 and 32 hours after starting exposures. With the higher concentrations there was a trend toward

increased mortality during exposure (near the end), and with intermediate concentrations there was a trend toward delayed deaths. At necropsy, the spleen most consistently showed evidence of toxic effects: Moderate to marked follicular phagocytosis was a frequent finding. Evidence of liver damage was rare, all sections of cardiac tissue appeared normal (Werner et al. 1943).

Dermal

Acute dermal toxicity of 2-ethoxyethanol in rabbits has proven to be low as considered on the basis of a dermal LD50 value of 3311-4576 mg/kg bw: Male albino rabbits were immobilized during a 24-hours skin contact period. Thereafter, the occlusive dressing was removed, and the animals were caged for the remainder of the 14-day total observation period. A dermal LD50 of 3.56 (2.24-5.66) ml/kg bw was stated for "Cellosolve" (Carpenter et al. 1956).

In a test using CELLOSOLVE Solvent (no data on purity), dermal LD50 values of 4.0 ml/kg (3720 mg/kg) bw for male rabbits and 4.92 ml/kg (4576 mg/kg) bw for female rabbits were detected using 3 groups of 5 males each and 3 groups of 5 females each (no further information on that doses). The test sample was dosed undiluted under impervious sheeting on the clipped, intact skin of the trunk. No skin reactions were observed; sluggishness, unsteady gait and prostration were noted. Most deaths occurred at 1-3 days, but one male dosed at 2.0 ml/kg died on day 13 after dosing. At necropsy, most victims and survivors demonstrated no unusual gross pathologic findings (Union Carbide Corp. 1983, unpublished report).

Human data:

Only data on acute oral toxicity of mixtures of toxic substances containing 2-ethoxyethanol are available.

Acute toxicity in humans has been observed after oral uptake of 50-200 ml 2-ethoxyethanol. This means that a range of about 1 to 30 mg/kg of body weight may by toxic to humans.

In 10 cases one death and, in two ones severe toxic effects were noted (5). Two phases have been described after intoxication with 2-ethoxyethanol: after a first phase shortly after ingestion a second phase has been observed appearing after a lag time of about 3-18 showing severe toxic effects by the GI-tract, CNS, lung and heart (Bonitenko 1990; Fucik 1969).

Conclusion:

Human data are only available for acute oral toxicity of mixtures of toxic substances containing 2-ethoxyethanol. In animals the acute toxicity of the substance is low as considered on the basis of oral LD50 values for rats of 2300-4700 mg/kg body weight, inhalative LC50 values for rats of 15.2 mg/l/4 hours and 7.36 mg/l/8 hours and dermal LD50 values of 3311-4576 mg/kg bw for rabbits. These data do not support existing classification and labelling. In consequence, R 20/21/22 should be removed.

4.1.2.3 Irritation

Animal data:

In a Draize skin irritation test with 6 rabbits CELLOSOLVE Solvent (no data on purity) produced no oedema, but minor to moderate erythema and minor desquamation: A 4-hour occlusive exposure to 0.5 ml of the substance resulted in minor to moderate erythema with average scores of 0.8 as maximum on the skin of 5/6 rabbits and minor desquamation on the skin of all rabbits within 2-7 days. The erythema were observed on 4 animals within 1 hour after the end of the contact period and persisted till day 2 on only 1 rabbit. At 7 days, minor erythema was observed on 1 rabbit with desquamation on 4 rabbits (Union Carbide Corp. 1983, unpublished report).

No skin irritation was observed in a test with rabbits according to EU guidelines: Skin irritation was tested with male and female albino rabbits by means of an exposure chamber containing a patch soaked with 0.5 ml of liquid substance or dilution's thereof for an exposure time of 4 hours. The limit concentrations for skin irritation were obtained by testing 5 different dilution's ranging from undiluted (100%) to 5%. The highest tested concentration at which the substance is not considered an irritant to the skin according to EU criteria is regarded as the limit concentration. Undiluted 2-ethoxyethanol proved to cause no skin irritation at all (Jacobs et al. 1987).

Moderate eye irritation which reversed within a 10-days observation period was observed in a Draize eye irritation test with CELLOSOLVE Solvent (no data on purity): A volume of 0.1 ml of the substance produced moderate diffuse corneal injury (mean scores of 1.2/0.7/0.5 for observations at 24 h/48 h/72 h), moderate iritis (mean scores of 1.0/0.3/0.3) and moderate to severe conjunctival irritation (mean scores of 2.8/2.2/1.1 for erythema and of 2.7/1.5/0.7 for oedema) in 6/6 rabbit eyes. After 48 hours, most ocular effects were beginning to subside, but necrosis developed on the nictitating membranes of two eyes. By 72 hours, two eyes had healed, five eyes had normal appearance after 7 days. All eyes healed within a 10-days observation period. Draize tests with 0.01 ml and 0.005 ml of the substances revealed similar irritation healing within 7 days (0.01 ml) and 3 days (0.005 ml) (Union Carbide Corp. 1983, unpublished report).

In a Draize test with rabbits "Cellosolve" proved to be a moderate eye irritant, injury grade 3 on a scale of 10 was detected in rabbit eyes. Grade 3 signifies that 0.5 ml of the undiluted substance yielded a score of over 5.0 and 0.1 ml yielded a score not over 5.0. Score 5 is defined as necrosis on 63-87% of cornea, a symptom visible after fluorescein staining. The reversibility of this observed eye lesion was not assessed. Ethanol demonstrated the same scoring result and was classified similar to 2-ethoxyethanol (Carpenter and Smyth 1946).

In Draize tests on rabbit eyes carried out in 1971, mild eye irritation was seen, but no information on lesions like pannus or other serious damage to eyes are recorded: Twenty-five laboratories submitted data on skin and eye irritation for 12 test substances within an intra- and interlaboratory collaborative study. Eye irritation was tested by means of a reference method and of nonreference methods being commonly used in each individual laboratory. The reference method used 0.1 ml of the test material tested in three left and three right eyes of male albino rabbits for each sample. The eyes were not washed following instillation. The eyes were examined and the degree of irritation recorded at 1, 24, and 72 hours and at 7 days after application of the sample. Observations on any animal were discontinued after 24 or 72

hours if the eyes were free of irritation. Injuries such as pannus, keratoconus, and sloughing of corneal epithelium were not included in the Draize scoring system. Within this study, 2-ethoxyethanol caused only slight transient eye irritation as considered on the basis of the recorded scores. Eye lesions other than irritation or the reversibility of detected lesions are not recorded (Weil and Scala 1971).

Human data:

Human data on local irritation or corrosion caused by 2-ethoxyethanol are not available.

Conclusion:

Human data on local irritation or corrosion caused by 2-ethoxyethanol are not available. In a Draize test with rabbits the substance caused mild skin irritation that reversed within 7 days. Draize eye tests with rabbits demonstrated moderate eye irritation that reversed within 10 days. Based on the results of the mentioned Draize tests, local irritant properties of the substance need no classification as „irritant“ according to EU guidelines.

4.1.2.4 Corrosivity

Based on the properties described in 4.1.2.3 2-ethoxyethanol is considered to be not corrosive.

4.1.2.5 Sensitisation

Animal data:

No skin sensitization was observed in a Magnusson Kligman test that was conducted according to OECD criteria. Intradermal induction concentration was 10% in saline and topical induction (after pre-treatment with 10% sodium laurylsulfate) was 100% as well as a challenge concentration of 100% (Hüls 1992).

Human data:

Human data on sensitization caused by 2-ethoxyethanol are not available.

Conclusion:

Human data on sensitization caused by 2-ethoxyethanol are not available. In a Magnusson Kligman test with guinea pigs no skin sensitization was observed.

4.1.2.6 Repeated dose toxicity

Animal data:

A number of repeated dose toxicity studies on 2-ethoxyethanol are available, with investigations performed in rats, mice, rabbits, and dogs. The major metabolites of 2-ethoxyethanol are 2-ethoxyacetic acid, ethoxyacetyl glycine and carbon dioxide. 2-Ethoxyethanol and its acetate 2-ethoxyethyl acetate have 2-ethoxyacetic acid as a common metabolite. It is created by oxidation of the free primary hydroxyl group of 2-ethoxyethanol by alcohol dehydrogenase and is finally excreted in urine (Illing and Tinkler 1985). The toxicity of 2-ethoxyethanol and 2-ethoxyethyl acetate in animals is based on the common metabolite, 2-ethoxyacetic acid.

Repeated administration of 2-ethoxyethanol by oral and inhalation routes in various experimental animals produced adverse effects on the blood and hematopoietic systems. Testicular atrophy, leading to cessation of spermatogenesis occurred in male rats, mice, rabbits and dogs following exposure to 2-ethoxyethanol through the inhalation and oral routes, and by subcutaneous injection.

Inhalation experiment:

14-day study (rat)

Effects of repeated inhalation of vapours of industrial solvents on animal behaviour were investigated in a short-time study with female rats (8-10/group). Vapour of 2-ethoxyethanol have been inhaled by rats selected by successful training performance, and effects on conditioned avoidance-escape procedure have been assessed. Conditioned avoidance-escape behaviour was studied by a modidation of the pol-climb method. Behavioural criteria were the abolishment or significant deferment of avoidance response (conditioned or buzzer response) and escape response (unconditioned or buzzer + shock response). Rats were trained to respond to both stimuli within two seconds and defermerment of avoidance or escape responses of greater than six seconds was considered significant. No other parameters with respect to sign of toxicity were documented in this study. Rats (CFE) were exposed in 200-liter chamber at approximately 95 litres/minute under slight negative pressure (whole body exposure) to 0, 500, 1000, 2000, and 4000 ppm (0, 1870, 3740, 7480, 14960 mg/m³) for 4 hours/day, 5 days/week for 2 weeks (10 exposure days). In this behavioural study, body weights of animals exposed to 4000 ppm 2-ethoxyethanol were significant reduced throughout the treatment period. A transient effect on body weight was noted during the first

three days of the study in the rats inhaling 2000 ppm 2-ethoxyethanol. 2-Ethoxyethanol did not affect the behavioural system at any time during the study.

Overall, repeated daily inhalation of 2-ethoxyethanol vapour at doses of 500-4000 ppm over a period of 2 weeks produced no relevant toxic effects in rats. No inhibition of conditioned avoidance-escape behaviour was manifested at any time during the study (Goldberg 1964).

5-week study (rat)

23 adult male Wistar rats were exposed in 400-liter chambers, through which air was passed at approx. 50 litre per minute (whole body exposure) to a calculated concentration of 1.37 mg/l (equal to 370 ppm) 2-ethoxyethanol vapour, for 7 hours/day, 5 days/week for 1 to 5 weeks followed by a 1 or 3 weeks recovery period. As controls, 23 additional rats were exposed to similar conditions in an exposure chamber, except for the absence of solvent vapours. Hematology was examined before, during, and after the five weeks of exposure. The determinations consisted of red and white cell counts, differential counts, reticulocyte counts, and hemoglobin estimations. Eight rats were used only for pathological examination. They were sacrificed in pairs, 1, 3, and 5 weeks after starting exposures, and 1 week after terminating exposures. The remaining 15 animals were used for growth and hematological studies. Three weeks after terminating exposures, after final observations on their blood, these animals too were sacrificed and tissues were prepared for microscopic examination. Histopathology from selected tissues and organs was reported.

There were no increase in mortality and no effects on body weights followed repeated exposures of rats to 1.37 mg/l 2-ethoxyethanol vapour. The study of the hematological findings revealed only slight but measurable effects on cellular elements of the blood. A week of exposure resulted in an increase in the percentage of juvenile granulocytes in the circulating blood. An increase of hemosiderin deposits and a decrease in content of lymphatic tissue in the white pulpa were noted in the spleen. The hemosiderin amount was not removed in the 3-week interval following termination of the exposure. A decrease of myeloid cells was noted during the periods following exposure, a finding that considered as evidence of toxic action on the blood forming elements in the spleen of rats. Fat replacement, slight to moderately marked in amount, in the bone marrow in the middle of the femoral shaft, was also noted in some animals three weeks following exposures. In no instance was it observed in controls. The only conspicuous finding in the liver was a variation in the density of the cytoplasm of the cells. It was suggested that this finding may indicate an endosmosis of fluid in the liver cells. No changes were reported in kidneys, heart or lungs, and the other tissues examined.

In summary, adult male Wistar rats exposed to 1.37 mg/l (equal to 370 ppm) 2-ethoxyethanol vapour for 7 hours/day, 5 days/week for 5 weeks, demonstrated no significant effects in red cell counts and on hemoglobin concentration. The increased percentage of juvenile granulocytes in the circulating blood is perhaps the result from increased destruction of erythrocytes. In addition, hemosiderin content and decrease in myeloid cells in the spleen, fat replacement in the bone marrow inferred a mild hematotoxic effect of 2-ethoxyethanol (Werner 1943).

13-week studies (rat and rabbit)

In an OECD 413-like study (histopathology from selected tissues and organs) the effects of 2-ethoxyethanol vapour were investigated in rats and rabbits following subchronic inhalation exposure. Sprague-Dawley CD rats (15/sex/group) and New Zealand White rabbits (10/sex/group) were used in the study. Animals were exposed in 10 m³ stainless steel and glass chambers (whole body exposure) to 0, 25, 100 or 400 ppm 2-ethoxyethanol vapours (equal to 0, 92.5, 390, or 1480 mg/m³) for 6 hours/day, 5 days/week for 13 weeks. 2-ethoxyethanol produced no mortalities in both species. The growth of both male and female rabbits was depressed slightly compared to the controls although a clear dose response was evident only at 400 ppm. Male and female rabbits exposed to 400 ppm 2-ethoxyethanol sustained decrease in hematocrit, hemoglobin concentration and erythrocyte count. No hematological changes were observed to either sex at lower concentrations. The only finding noted in rats was a reduced leucocyte count in females exposed to 400 ppm, while this same parameter was statistically significantly increased for the 25 and 100 ppm dose group males relative to control. There were no treatment-related effects on the biochemical parameters as well as on the urinary status in rats. Total serum protein was elevated slightly in male rabbits exposed to 400 ppm. Serum cholesterol was reduced in all groups of treated rabbits of both sexes exposed to 400 ppm. In rats, the only organ weight changes consisted of a decrease in absolute and relative pituitary weight in males exposed to 400 ppm and a decrease in absolute spleen weight at all concentrations in females. The decrease in relative spleen weight was significant only at 100 and 400 ppm. The testes weight of rabbits aswas decreased significantly at 400 ppm. Microscopic examination in animals of the 400 ppm-group showed testicular lesions in 3 out of 10 rabbits characterized by slight focal degeneration of the seminiferous tubular epithelium.

The results from this study indicate that the rabbit is the more sensitive species to subchronic exposure to 2-ethoxyethanol vapour. Data presented support a dose-related effect to both sexes of this animals exposed to 400 ppm. Animals at this concentration showed evidence of slight anemia and testicular lesions characterized by degeneration of tubular epithelium. The anemia observed in rabbits appears to be the result of an increase in destruction of erythrocytes rather than a depression of erythropoiesis.

The concentration at which no toxicological significant effects are observed from 13-week inhalation exposure to 2-ethoxyethanol is 400 ppm (1480 mg/m³) for rats.

For rabbits, a NOAEC for male reproductive effects was judged at 100 ppm (390 mg/m³) 2-ethoxyethanol (Bio/dynamics Inc. 1983; Barbee 1984).

12-week study (dog)

In order to determine effects of exposure on the blood and on the kidney function two dogs were exposed to a vapour concentration of 0, or 840 ppm (3091 mg/m³) 2-ethoxyethanol. Dogs were exposed in glass chambers (whole body exposure) 7 hours/day, 5 days/week for 12 weeks followed by a 5 week recovery period. The study design did not follow OECD or EEC

methods. To determine effects of exposure on the blood erythrocyte, reticulocyte, leucocyte, and differential counts, and hemoglobin and hematocrit concentration were examined. These determinations were made for selective times during the exposure and in the post-exposure period. During the course of exposure, indications of central depression or stimulation were not observed. At the termination of exposure animals appeared to be in good physical condition. Erythrocytic indices (hemoglobin and hematocrit concentrations, and erythrocyte counts) were marginally reduced and microcytosis, hypochromia and polychromatophilia were observed in the erythrocytes. The blood were characterized by a greater than normal number of the immature white cells at the end of week one. An increase in the number of juvenile granulocytes noted between one and 8 weeks. In general, these findings appeared a few weeks after starting exposure and the alterations of erythrocytes persisted throughout the course of exposure. With regard to the effect of the glycol ethers on the kidney function, it appears that conditions of these experiments did not influence urine output. Calcium oxalate crystals were often detected in the urine. No other relevant findings were observed in urinary sediments. No histopathological abnormalities were detected in the lungs, liver, kidneys, spleen, heart, urinary bladder, pancreas, and large and small intestines (gonads were not examined). There was no evidence of bone marrow injury. A lack of such damage is indicated by the observation of increased numbers of immature granulocytes during exposures, and the observation of considerable polychromatophilia at the end of the exposures. In the dogs, there was little indication that 2-ethoxyethanol altered the red blood cells through a hemolytic action.

Exposure of dogs 7 hours/day, 5 days/week for 12 weeks to 840 ppm (3091 mg/m³) 2-ethoxyethanol resulted in decreased haemoglobin and hematocrit concentrations, and erythrocyte counts. Red blood cells showed an increased hypochromia, polychromatophilia, and microcytosis. The white cell picture was characterized primarily by a shift to the left (Werner 1943).

Additional information from reproductive studies: (rat)

This study was designed to evaluate subtle behavioural changes which might result from exposure to 2-ethoxyethanol during the gestation period in rats via inhalation. Some further information on reproductive effects is described in section 4.1.2.9.

Behavioural parameters, and neurochemical deviations on offspring from dams exposed to 2-ethoxyethanol on gestations days 7-13 or 14-20 were studied. There were no hematological and/or biochemical parameters, and no histopathology available. In a pilot dose-finding study, pregnant rats were exposed to concentrations of 0, 100, 200, 300, 600, 900, and 1200 ppm of 2-ethoxyethanol for 7 hours/day during gestation days 7-13 or 14-20. No offspring survived inhalation exposures of 900 ppm. There were approx. 34% neonatal deaths even after prenatal exposure to 200 ppm. In the main study of this experiment pregnant rats were exposed to 100 ppm 2-ethoxyethanol under similar exposure conditions on gestation days 7-13 or 14-20. Six behavioural tests (ascent, rotorod, open field, activity wheel, avoidance conditioning, operand conditioning) were selected to measure various CNS functions at several stages of development. The tests were selected to evaluate motor, sensory, and cognitive functions.

Behavioural testing of offspring of mothers exposed to 100 ppm 2-ethoxyethanol on gestations 7-13 revealed: impaired performance on a rotorod test of neuromuscular ability;

prolonged latency of leaving the start area of an open field; and marginal superiority in avoidance conditioning begun on day 34 of age. Offspring from dams exposed to 2-ethoxyethanol on gestation days 14-20 were less active than controls in a running wheel, and received an increased number and duration of shocks in avoidance conditioning begun on day 60 of age. Neurochemical evaluation of whole-brain samples from newborn pups revealed significantly decreased levels of norepinephrine in offspring from both exposure periods. In regional analyses of brains from 21-day-old offspring of dams exposed to 100 ppm 2-ethoxyethanol on gestation days 7-13, the cerebrum had significant elevations in acetylcholine, norepinephrine, and dopamine; the cerebellum had nearly a 3-fold increase in acetylcholine; the brainstem had an increase in norepinephrine; and the midbrain had excesses of acetylcholine, norepinephrine and protein. In brains of 21-day-old offspring of dams exposed to 2-ethoxyethanol on gestation days 14-20, the cerebrum had significant elevations in acetylcholine, dopamine, and 5-hydroxytryptamine.

Overall, the results indicated that there are behavioural and neurochemical alterations in offspring of rats following prenatal exposure to 100 ppm 2-ethoxyethanol. A NOAEC for these effects did not demonstrate (Nelson 1981).

Oral: rat, mouse, dog

2-Ethoxyethanol administered by oral routes (by gavage, with the diet or drinking water) produced a marked toxic effect on the testes and the hematopoietic system.

Gavage studies

2-day study (rat)

Smialowicz et al. (1992) studied the effects of various glycol ethers on the immune function in adult male Fischer 344 rats. Six rats per group were administered orally by gavage with 0, 50, 100, 200, or 400 mg/kg bw/day 2-ethoxyethanol or 2-ethoxyethyl acetate in water for two consecutive days. To generate an antibody response, the rats were immunized in vivo on treatment 4 hours before two treatments with either the sheep erythrocyte antigen or the trinitrophenyl-lipopolysaccharide antigen. The primary plaque-forming cell response to trinitrophenyl-lipopolysaccharide on day 3 following immunization used to evaluate the immunotoxic potential of test substances was comparable in treated and control animals. No data on hematology, clinical chemistry parameters and histopathology.

Oral dosing of adult rats to 2-ethoxyethanol or its principal oxidative metabolite 2-ethoxyacetic acid failed to alter the antibody response to trinitrophenyl-lipopolysaccharide.

11-day studies (rat)

In a study to establish the temporal development and to identify the primary cell site of testicular lesions, 36 young male Sprague-Dawley rats/group were administered orally with 0, 250, 500, or 1000 mg/kg bw/day 2-ethoxyethanol for up to 11 days. Six animals from each

group were killed after 6 and 24 hr, and after 2, 4, 7, or 11 days of treatment. No data on hematology and clinical chemistry parameters were available. At necropsy, the testes, seminal vesicles, prostata and liver were examined and weighed. The testes, epididymis and liver were examined by light or electron microscopy. Testis weights were significant reduced after 11 days at 500 mg/kg bw/day by 67.2% of control and at 1000 mg/kg bw/day by 84.4%, respectively. Seminal vesicle weight was also significant reduced (57.8%) at this time at 1000 mg/kg bw/day only. Prostate weight was not affected by treatment. Oral dosing of rats with 2-ethoxyethanol for 1-11 days resulted in a dose-related decrease in sperm count and changes in sperm motility and morphology at dose levels at 500 mg/kg bw/day or more. In this groups there was a testicular damage consisting of a degeneration of the later stages of primary spermatocytes and secondary spermatocytes. A partial depletion and maturation arrest of early stages of spermatids were also seen. Dosing of rats at 250 mg/kg bw/day 2-ethoxyethanol for 11 days produced no testicular abnormalities. Microscopic examination (light or transmission electron microscopy) of the liver revealed no effect at any dosage (Foster 1983).

eeThese findings were confirmed in a similar set of experiments using 2-ethoxyethanol in which testicular lesions were examined at sequentially timed intervals (6 h, 1, 2, 4, 7, and 11 days) during the dosing period of 11 days. 2-Ethoxyethanol exerted no adverse effect at 250 mg/kg bw/day, but it did at doses of 500 and 1000 mg/kg bw/day. Although no testicular abnormalities were observed in any of the groups 6 hours after dosing, degenerative spermatocytes were frequently seen 24 hours after dosing with 2-ethoxyethanol. Dose levels of 500 and 1000 mg/kg bw/day produced degeneration of later stage of the pachytene development. Although the 500 mg/kg bw/day 2-ethoxyethanol induced a more extensive lesion than did 1000 mg/kg bw/day after 48 hours of dosing, this trend was reversed with prolonged dosing. It was concluded that primary spermatocytes undergoing pachytene development constitute the initial and major site of morphologic damage (Foster 1984).

The no adverse effect level over the 11-day treatment period was 250 mg/kg bw/day for 2-ethoxyethanol (Foster 1983, 1984).

6-week study (rat)

In a further study (Hurtt and Zenick, 1986) with an active schedule of copulation employed to reduce cauda epididymal sperm reserves in the rat, any effects using 2-ethoxyethanol were investigated in the actively copulated males in comparison to the nonmated animals. Adult male Long-Evans rats were assigned to a “mate” or “non-mate” condition, with the former mated every other day (3-hr session) for 2 weeks prior to and then throughout the experiment. After two weeks, males from each group were randomly assigned to receive either 0 150, or 300 mg/kg of 2-ethoxyethanol by oral gavage for 5 days/week for 6 weeks. Males were then sacrificed and organ weights, testicular spermatid counts, and cauda epididymal sperm count and sperm morphology were obtained. 2-Ethoxyethanol produced a significant reduction in testicular weight and spermatid counts in mated and nonmated animals receiving 300 mg/kg. Significant decreases were also noted in epididymal sperm count and percentage of normal morphology. However, these effects were seen in the *nonmated* animals only at 300 mg/kg, whereas significant reductions in epididymal sperm count and percentage of normal morphology were also obtained at 150 mg/kg/ in the males mated bidaily. Thus, the data from

this study using 2-ethoxyethanol as a model compound suggested, that bidaily mating, by reducing the epididymal sperm reserves, may enhance the detection of spermatotoxicity.

13-week study (rat)

The study design of the described study did not follow OECD or EEC methods (as for number of animals per dose groups, dose regime, no details on hematology and biochemistry parameters, only data of hematocrit and hemoglobin). Groups of 5 Wistar rats per sex were dosed daily by gavage 0, 46 or 93 mg/kg bw/day for 13 weeks or 93 mg/kg bw/day for 59 days followed by an oral dose of 372 mg/kg bw/day for the remainder of the 13 week period. No adverse effects were observed in these dose groups. The dose level of 186 mg/kg bw/day caused beginning adverse effects, consisting reduced hemoglobin content and hematocrit values. Following oral administration of 186 mg/kg bw/day for 13 weeks, the testicular interstitium was occasionally broken down edematously, and there was a hypospermia in the canals. The oral administration of 186 mg/kg bw/day 2-ethoxyethanol for 59 days, followed by oral administration of 743 mg/kg bw/day for the 32 days remaining in the 13-week period, caused similar testicular changes. Hemosiderin accumulation and isolated hematopoietic foci were observed in the spleen of all 2-ethoxyethanol-dosed rats. Histological investigations performed in liver, kidney, heart, lungs, adrenal and thyroid glands, pancreas, stomach, and intestinum revealed unspecified microscopical findings at all dose levels.

The no adverse effect level over the 13-week treatment period was established at 93 mg/kg bw/day (Stenger 1971).

5-week study (mouse)

In a 5-week oral study (report in Japanese, only summary in English) JCL-ICR mice (5 males/group) were given various doses (0, 500, 1000, 2000, or 4000 mg/kg bw/day) of 2-ethoxyethanol and 2-ethoxyethyl acetate in aqueous solution 5 days/week for 5 weeks. Histopathology from selected tissues and organs was reported. In this investigation on hematological and testicular effects, all mice receiving 4000 mg/kg bw/day 2-ethoxyethanol died before completion of the treatment period. At 2000 mg/kg bw/day, significantly reduced white blood cell counts compared with control values were noted. No disturbances of erythrocytic parameters were observed following administration of 500 to 2000 mg/kg bw/day. At 2000-4000 mg/kg bw/day doses of 2-ethoxyethanol, marked testicular atrophy was produced and was assumed in terms of testicular weight, both absolute and relative to body weight. Statistically significant decrease of the testicular weights of exposed animals in comparison to control animals were noted in those given doses of at least 1000 mg/kg bw/day. Histologically, varying dosage-related degrees of seminiferous tubule atrophy were observed. In the 2000 mg/kg bw/day group, the diameter of the seminiferous tubules decreased, spermatozoa and spermatids completely vanished, and spermatocytes existed in extremely small numbers in only some of the tubules; interstitial tissue also increased.

Testicular atrophy occurred in mice given oral doses of $\geq$1000 mg/kg bw/day 2-ethoxyethanol, for 5 days/week during a 5-week period. For mice, the NOAEL noted in this

study for effects on the blood and male reproductive system was 500 mg/kg bw/day (Nagano 1979).

103 week studies (rat and mouse)

In the first range-finding study Fischer 344/N rats and B6C3F1 mice were given 2-ethoxyethanol in drinking water at dose levels ranging from 200 to 1600 mg/kg bw for rats and 400 to 2800 mg/kg bw for mice for 14 days. There was a depression in weight gain in rats at all dose levels and a concomitant decrease in water consumption. Because of concern that a palatability problem might obscure any toxicity due to the chemical treatment, the route of administration was changed from dosed water to gavage in a water vehicle.

In the second range-finding study groups of five male and five female rats and mice received 2-ethoxyethanol by oral gavage five times per week for 2 weeks in doses of 0, 0.3, 0.6, 1.25, 2.5 and 5.0 g/kg bw. In each of these both studies, there was 100% mortality at the 5.0 g/kg bw level. No mortality occurred in rats and mice at the 1.25 g/kg bw dose level.

Groups of 50 rats and 50 mice of both sexes were administrated 2-ethoxyethanol dissolved in deionized water by gavage five times per week for 103 consecutive weeks at dose levels of 0, 500, 1000, and 2000 mg/kg bw/day. This was followed by a one-week observation period. Data on hematology and/or clinical chemistry were not available. Repeated administration of 2-ethoxyethanol at the 2000 mg/kg bw/day dose level was lethal to rats and mice. Early mortality in the high-dose groups of both species and sexes appeared to be due to stomach ulcers. As a consequence of the high mortality rate, the 2000 mg/kg bw per day dose was terminated at week 17 to 18. Testicular atrophy was observed in male rats that died early in this study and in the medium- and high-dose male mouse groups (≥500 mg/kg bw/d). Gross lesions noted at necropsy indicate that the testes of the 2000 mg/kg bw/day dosed mice were generally decreased in size. There were no gross lesions seen in the 2000 mg/kg bw/day-dose female mice. At necropsy, there was an increased incidence of enlarged adrenal glands in male rats treated with 500 or 1000 mg/kg bw/day 2-ethoxyethanol in comparison to control male rats. Overall, 2-ethoxyethanol caused testicular atrophy in male rats and mice. This effect was apparent in the 2000 mg/kg bw/day-dose male rats which died early in the 2-year study and in the 1000 and 2000 mg/kg bw/day-dose male mice. Chronic treatment with 500 or 1000 mg/kg bw/d 2-ethoxyethanol induced an apparent enlargement of the adrenal gland in male rats. Based on the data presented a NOAEL could not establish for rats and mice (Melnick 1984).

13-week study (dog)

Daily oral administration by gelatine capsule of 0, 46, or 93 mg/kg bw/day 2-ethoxyethanol for 13 weeks to Beagle dogs (3 males and 3 females/groups) had no adverse effect. Oral administration of 186 mg/kg bw/day decreased hemoglobin level and hematocrit values after 5, 9 and 13 weeks (no other hematology or clinical chemistry parameters were examined). Histopathology from selected tissues and organs was reported. Testicular changes, including apparent disruption of spermatogenesis, were seen in all males at this dosage. In one dog, the lumen of the tubuli appeared to be expanded, and the last maturation stages of the seminal epithelium clearly were absent in many of the tubuli. In the second dog, tubuli were

constricted, and parent and powdery spermatophores had been retained. In the third dog, there was conspicuous flattening of germinal epithelium with complete absence of upper layers; the parent epithelium was, in some cases, absent in these tubuli. Slight kidney lesions were observed in two males and one female; the lumen in the region of the tubuli contorti was expanded, and the epithelium was flattened. Hemosiderin accumulation and isolated hematopoietic foci were observed in the spleens of all dogs.

No adverse effects were seen in dogs at 93 mg/kg bw/day (Stenger 1971).

Diet studies

90-day study (rat)

During the 90-day study, groups of 10 male and 10 female Dow-Wistar albino rats were given diet containing 0, 0.01, 0.05, 0.25, or 1.25% 2-ethoxyethanol (average 2-ethoxyethanol consumption: 0, 8, 39, 190, 1007 mg/kg bw/day in males and 0, 9, 45, 222, 1039 mg/kg bw/day in females). The study design did not follow strictly the OECD 408. Data on hematology and/or clinical biochemistry tests were not available. Organ weight only from the liver and kidneys of each rat were reported. At sacrifice, the organs were examined for any signs of pathology or infections and urinary bladder were examined for concretions. There were no treatment-related effects on mortality. Diet consumption was consistently lower than the controls for all treated groups. The body weight gain was significantly depressed throughout the study in male rats fed 1.25% 2-ethoxyethanol. The same effect was apparent in the females but it was not statistically significant. None of the mean liver or kidneys weights of the male and female rats differed significantly from those of their controls.

Histopathological investigations performed on numerous tissues of organs revealed unspecific changes at all dose levels which were not related to the treatment. No histomorphological abnormalities in the testis were found at the 1.25% dose level.

The results of feeding of 2-ethoxyethanol in the diet of rats for three months showed that 1.25% (about 1000 mg/kg bw/day) 2-ethoxyethanol in the diet depressed body weight gain of male rats. Furthermore, it is concluded that 0.25% (about 200 mg/kg bw/day) 2-ethoxyethanol in the diet over a period of 90 days produced no significant effects in rats (Union Carbide 1962).

2-years study (rat)

In a chronic oral toxicity study marked testicular enlargement, interstitial edema and tubular atrophy were seen in two-thirds of a group of rats fed 1.45% (equivalent to approx. 900 mg/kg bw/day) 2-ethoxyethanol in their diet for 2 years. No data on hematology and clinical chemistry parameters were available. Histopathology from selected tissues and organs was reported. The testicular lesions were more often bilateral than unilateral and consisted of marked interstitial edema and marked tubular atrophy. In a few rats slight chronic renal lesions consisted of tubular atrophy with less glomerular atrophy; tubular casts, usually hyaline, and slight degrees of lymphocytic infiltration and fibrosis were reported (Morris 1942).

Drinking water studies

In a National Toxicity Program (NTP) study (1993), comparative toxicity studies with three glycol ethers, one of them was 2-ethoxyethanol, were conducted in Fischer 344/N rats and B6C3F1 mice in both 2-week and 13-week drinking water studies. In addition, stop-exposure studies in male rats were performed.

Toxicological endpoints evaluated in animals included hematology, clinical chemistry, urinalysis, histopathology, and reproductive system parameters (some further information on reproductive effects is described in section 4.1.2.9).

2-week studies (rat and mouse)

Groups of five male and five female rats and mice received 2-ethoxyethanol in the drinking water in doses of 0, 300, 600, 900, 1500, or 2500 mg/kg bw for 14 days. Complete histopathologic examinations were performed only on those organs showing gross evidence of lesions.

Rat: There were no 2-ethoxyethanol-related effects on survival for rats. No clinical signs of toxicity were observed for males or females treated with 2-ethoxyethanol. There were dose-related decreases in mean water consumption for rats at each sex treated with 2-ethoxyethanol.

Based on the reduced water consumption, the ultimate compound uptake was estimated to be ranged from 200 to 1600 mg/kg bw/day. Decreased body weight gains were noted for both male and female rats treated with 2-ethoxyethanol. Excluding changes in thymus and testis weights, the majority of changes in absolute and relative organ weights for rats treated with 2-ethoxyethanol were related to low final body weights. Dose-related decreases were noted for the absolute and relative testis weights of males. There were no chemical-related gross lesions in male or female rats. At the end of the study, the testis and epididymis from all male rats were evaluated microscopically. Degeneration of the seminiferous tubules was present in male rats of the 1500 and 2500 mg/kg bw/day dose groups. At 2500 mg/kg bw/day, the severity of degeneration ranged from moderate to marked; at the next dose level, the severity of degeneration ranged from minimal to mild. No testicular effects were seen in male rats in the 300-900 mg/kg bw/day dose groups.

Mouse: There were no treatment-related effects on mortality in females. One male receiving the target dose of 900 mg/kg died on day 10. No clinical signs of toxicity were observed for males or females treated with 2-ethoxyethanol. Average water consumption was similar or somewhat increased for males in all treated groups excluding the 2500 mg/kg bw treatment group; average water consumption for males in this dose group and females in all treated groups was decreased. The estimated compound consumption based on water consumption by males and females ranged from 400 to 2800 mg/kg bw. The mean body weights and mean body weight changes of males and females treated with 2-ethoxyethanol did not differ from those of the control groups. Changes in organ weights for mice treated with 2-ethoxyethanol were minimal. For male mice in the 2500 mg/kg bw/day-dose group, relative testis weight

was significant lower than those of the controls. No chemical-related gross lesions were noted in males or females. Microscopic evaluation of tissues was not performed.

Overall, in the 2-week study in rats, decreases in relative thymus weights were noted for males and females at all dose levels. Generally, male and female mice treated with 2-ethoxyethanol for 2 weeks also exhibited decreases in relative thymus weights. Degeneration of the seminiferous tubules was present in male rats in the 1500 and 2500 mg/kg bw/day dose groups. In male mice at the same dose level, decreases in relative testis weights were seen.

13-week study (rat and mouse)

Rat: Groups of 10 male and 10 female rats received 2-ethoxyethanol in the drinking water at concentrations of 0, 1250, 2500, 5000, 10000, or 20000 ppm (average 2-ethoxyethanol consumption: 0, 109, 205, 400, 792 or 2240 mg/kg bw/day in males and 0, 122, 247, 466, 804, or 2061 mg/kg bw/day in females). Chemical-related mortality occurred in male and female rats administered 20000 ppm 2-ethoxyethanol. At 20000 ppm, five male and seven female rats died or were killed early; due to the high mortality at this exposure level, the remaining male and female rats in this group were removed from treatment during week 9 of the study. There were dose-related decreases in mean water consumption for rats at each sex treated with 2-ethoxyethanol. Decreased body weight gains occurred in all dosed rats. Clinical signs noted for male and female rats were emaciation, diarrhea, abnormal posture, and tremors. The hematological evaluation at week 1, 3, and 13 showed in male and female rats an anemia at $\geq$10000 ppm, as indicated by decrease in HCT and HGB concentrations and RBC count, and was macrocytic (increase in mean cell volume), hypochromic (decrease in mean hemoglobin concentration), and regenerative (decrease in reticolocyte count). Thrombocytopenia was moderate at each time point, and the moderate leukopenia (lymphopenia and neutropenia). Treatment-related histopathologic findings were observed in the thymus, testes, and hematopoietic tissues (spleen, bone marrow, and liver). At $\geq$2500 ppm, absolute and relative thymus weights were decreased in a dose-related fashion for males and females, significantly only in males. Microscopic examination of the thymus revealed atrophy at $\geq$10000 ppm in both males and females. Absolute and relative testis weights for males in the 10000 ppm group were significant lower than those of the control group. A reduction in testes size was noted in males in the 10000 and 20000 ppm groups. Testicular degeneration was observed in all male rats administered 2-ethoxyethanol at concentrations of $\geq$5000 ppm for 13 weeks. There was a dose-related degeneration of germinal epithelium in the seminiferous tubules of the testes. Histopathological lesions registering secondary effects to the anemia included increased hematopoiesis and hemosiderin pigmentation in the spleen, increased bone marrow hematopoiesis and hematopoiesis, and increased hemosiderin pigmentation in Kupffer's cells of the liver. Sperm morphology was performed on rats receiving 0, 2500, 5000, or 10000 ppm 2-ethoxyethanol. All spermatozoal measurements were significantly less than those of the control group for males in the 10000 ppm group, and sperm concentration was significant less than that of the control group for males treated with 2500 or 5000 ppm 2-ethoxyethanol.

In a special stop-exposure study in male rats (30 male Fischer 344/N rats/group) in which administration of 2-ethoxyethanol at dose levels of 5000, 10000, or 20000 ppm (average 2-ethoxyethanol consumption: 407, 792, 2390 mg/kg bw/day) was stopped after 60 days. At the end of the treatment period, 10 rats/dose groups were killed and examined for gross lesions. If

lesions were found at the 60-day necropsy, half of the remaining animals were killed after a 30-day recovery period, and the other half were killed after a 56-day recovery period. At necropsy, the testes and epididymides were removed. The right testis and epididymis were weighted, and the testes and the caput and cauda of the left epididymis were examined microscopically. 20/30 animals in the 20000 ppm group died or were killed before the scheduled 60-day evaluation. Due to the excessive mortality in males receiving 20000 ppm in both the stop-exposure and 13-week studies, the five surveying rats in the 20000 ppm base-study group were combined with the 10 surveying rats in the 20000 ppm stop-exposure group at Day 60 of the stop-exposure study. Microscopic examination revealed moderate to marked degeneration of the seminiferous tubules in rats treated with 10000 or 20000 ppm 2-ethoxyethanol but not in rats treated with 5000 ppm. At the 30 and 56 day recovery periods, there was no evidence of recovery from testis lesions in these groups. Although no degeneration was evident in the testis of rats from the 5000 ppm group when the exposure was stopped (day 60), minimal degeneration, similar to that seen at this dose level in the base study, was present in most male rats at the 30 (6/10) and 56 (7/10) day recovery periods.

In summary, 2-ethoxyethanol administration to rats caused early deaths (20000 ppm), decreased body weight gains (≥1250 ppm), thymus involution (≥2500 ppm), testicular atrophy (≥5000 ppm), hypospermia (≥2500 ppm), and hemolytic anemia (10000 ppm). TNmale reproductive effects was 1250 ppm (equivalent to 109 mg/kg bw/day). No adverse toxic effects were observed in female rats at 2500 ppm. Therefore, the NOAEL for female rats was estimated at 2500 ppm (equivalent to 205 mg/kg bw/d).

Mouse: Groups of 10 males and 10 females received 2-ethoxyethanol in the drinking water at concentrations of 0, 2500, 5000, 10000, 20000, or 40000 ppm/kg bw (average 2-ethoxyethanol consumption: 0, 587, 971, 2003, 5123, or 7284 mg/kg bw/day in males and 0, 722, 1304, 2725, 7255, or 11172 mg/kg bw/day in females). No premature deaths occurred in mice administered 2-ethoxyethanol. The males and females of the 20000 and 40000 ppm groups showed emaciation and lower body weight gains than those of control groups. Absolute testis weights were significant decreased for males in the 20000 and 40000 ppm dose groups. There also were some other changes in absolute and relative organ weights in dosed mice which were attributed to the low final mean body weights.

Treatment-related gross lesions consisted of small testes and epididymides in males from the 40000 ppm group. In this dose group, histopathological findings were present in the spleen and testis of males and the spleen and adrenal gland of females. In male mice, degeneration of the testis was characterized as a marked, diffuse loss of germinal epithelium in the seminiferous tubules. Histopathologic lesions were not seen in the testis of mice in the lower dose groups. In the spleen of females in the 20000 ppm group and males and females from the 40000 ppm groups, there was a minimal to mild increase in hematopoiesis; there was also a minimal increase in splenic hematopoiesis in one female mouse in the 10000 ppm group. Splenic hematopoiesis was characterized by an increase in the number of erythroid elements and megakaryocytes in the red pulp. Based upon histologic sections, there was no apparent effect in the bone marrow. At 10000 ppm, female mice had a hypertrophic zona reticularis with apparent lipid vacuolization of the cells in the adrenal gland.

In the sperm morphology study, values for sperm motility, spermatid heads per testis, and spermatid count were significantly lower than control values for males receiving 20000 ppm 2-ethoxyethanol.

In summary, mice showed reduced body weight gain, emaciation, and lower testes weight administered with ≥20000 ppm 2-ethoxyethanol.. Testicular atrophy was observed at 40000 ppm and dys/hypospermie at ≥20000 ppm. The NOAEL for all adverse effects of this 13-week study was 5000 ppm for mice.

Based on the data presented, the toxic effects following repeated oral administration of 2-ethoxyethanol were more severe in rats than in mice. The major target organs for toxicity were the testes in males of both species and the hematopoietic system in both sexes and species. Decreases in testicular and epididymal weights were seen in male rats and mice. Testicular atrophy was accompanied by lesions characterized by degeneration of the germinal epithelium in the seminiferous tubules of the testes, abnormal sperm morphology, and reduced sperm counts. Mild to moderate hemolytic anemia and corresponding effects such as extramedullary hematopoiesis was observed in male and female rats. In mice, minimal to mild increase in splenic hematopoiesis was noted. Furthermore, there was thymus atrophy in rats.

Overall, in the 13-week study in rats, the NOAEL for decreased thymus weights in males was 1250 ppm (109 mg/kg bw/day) 2-ethoxyethanol; for female rats, the NOAEL for all histopathologic and hematotoxic effects was 2500 ppm (205 mg/kg bw/day). The NOAEL for male reproductive effects was estimated in rats at 1250 ppm (equivalent to 109 mg/kg bw/day).

For male mice treated with 2-ethoxyethanol for 13 weeks, the NOAEL for testicular degeneration and increased hematopoiesis in the spleen was 20000 ppm (5123 mg/kg bw/day) and for female mice, the NOAEL for adrenal gland hypertrophy and increased hematopoiesis in the spleen was 5000 ppm (1304 mg/kg bw/day).

Dermal application:

There were no animal studies relating to dermal exposure.

Subcutaneous application: (rat)

4-week study (rat)

Histopathological testicular lesions were reported in Wistar rats (5/sex/group) treated subcutaneously with varying doses of 2-ethoxyethanol (0, 100, 200, 400, or 800 µl/kg bw/day, approx. 0, 93, 186, 372, or 744 mg/kg bw/day). Treatment of rats for 4 weeks with 372 mg/kg bw/day caused testicular damage consisting of maturation arrest of spermatogenesis, interstitial edema and polynuclear cell infiltration. In the female groups, a decrease in body weight gain was recorded at 186 mg/kg bw/day or more, in addition, a low food intake was seen in all animals receiving 744 mg/kg bw/day. Dyspnoea, somnolence and slight ataxia were noted in rats dosed with 372 and 744 mg/kg bw/day 2-ethoxyethanol. Subcutaneous administration of 744 mg/kg bw/day 2-ethoxyethanol caused occasional edema and hemorrhaging at the injection site. Microscopic examination of the liver revealed a dissociation of the lobular structure and small vacuoles in hepatocytes, and in the kidney swelling of the tubular epithelium at 372 mg/kg bw/day. The histopathological findings

described in the 372 mg/kg bw/day group were more pronounced in the 744 mg/kg bw/day group.

The NOAEL for all adverse effects was 186 mg/kg bw/day in males and 93 mg/kg bw/day in females (Stenger 1971).

Intravenous application: (dog)

22-day study (dog)

Groups of dogs (2/sex) given 2-ethoxyethanol intravenous at levels 93 and 465 mg/kg bw/day 5 days/week for 22 days developed local irritation at the injection site and ataxia. Treatment with 465 mg/kg bw/day caused pronounced thrombophlebitis. No change in hemoglobin levels or hematocrit values (no other hematology or clinical biochemistry parameters were examined) and no pathological organ findings of the 13 organs examined inclusive gonads, spleen, and thymus were noted. Data from this study was evaluated as further information (Stenger 1971).

In the following chapter, data on toxic effects of repeated exposure to 2-ethoxyethanol from animal studies with respect to target organs and systems were summarized.

Summary of repeated dose toxicity in animals with respect to target organs and systems

Effects on the blood and hematopoietic system

Hematotoxic effects were observed in a number of species following repeated administration of 2-ethoxyethanol by oral, and inhalation routes.

Inhalation experiments:

Prolonged inhalation of 2-ethoxyethanol caused anemia in rabbits exposed to >400 ppm (6 h/d, 5 days/week, 13 weeks) (Barbee 1984; Bio/dynamics Inc 1983) and minimal signs of anemia in dogs exposed to 840 ppm (7 h/d, 5 days/week, 12 w) (Werner 1943). The inhalation studies on rats did not result in obvious anemia, but increased hemosiderin depositions in the spleenic red pulp gave indications on an increased cleavage of damaged erythrocytes. Besides the effects on the red cell compartment, there were also effects on the leukocytes consisting of an increase of immature granulocytes presumably represent an inflammatory reaction to other target effects. The decrease of myeloid cells in the spleen and the bone marrow involution reported in adult rats exposed to 370 ppm (7 h/d, 5 d/w, 5 w) (Werner 1943) give hind on a possible immunsuppressive effect of 2-ethoxyethanol. However, eethese findings were not confirmed by any other study.

Oral administration:

There are several oral studies which consistently reported anemic effects due to 2-ethoxyethanol administration. An increased deposition of hemosiderin in the spleen (and the liver) indicated hemolysis as a cause of anemia, the increased hematopoiesis at extramedullary sites demonstrated the regenerative capacity of the erythropoietic compartment.

Reduced hemoglobin concentrations and packed cell volumes were noted in rats which had received 93 or 186 mg/kg bw/day of 2-ethoxyethanol orally for the first 8 weeks of a 13-week study, followed by 370 and 741 mg/kg bw/day respectively for the remaining 5 weeks. In rats which had received 186 mg/kg bw/day, either for 8 weeks (followed by 743 mg/kg bw/day for 5 weeks) or for the full 13 weeks, increased splenic hemosiderin was noted on histopathological examination. No effects were seen in rats receiving $\leq$93 mg/kg bw/day 2-ethoxyethanol for 13 weeks (Stenger 1971).

Reduced hemoglobin level and hematocrit values were evident in Beagle dogs when given daily 186 mg/kg bw/day 2-ethoxyethanol for 13 weeks (Stenger 1971).

The oral administration to mice at a dosage of 2000 mg/kg bw/day, 5 days/week for 5 weeks did not result in any disturbances of erythrocytic parameters, although reduced white blood cell counts were noted. All mice treated with 4000 mg/kg bw/day 2-ethoxyethanol died before blood sampling. No effects on the peripheral blood were noted following administration of 500 or 1000 mg/kg bw/day (Nagano 1979).

In 13-week drinking water studies in F344/N rats and B6C3F1 mice, at 10000 ppm respectively 20000 of 2-ethoxyethanol hemolytic anemia was observed. For female rats treated with 2-ethoxyethanol for 13 weeks, the NOAEL for hematotoxic effects was 5000 ppm (466 mg/kg bw/day). For female mice, the NOAEL for increased hematopoiesis in spleen was 5000 ppm (1304 mg/kg bw/day) (NTP 1993).

Summary of hematotoxic effects in animals following repeated administration of 2-ethoxyethanol are presented in Table 4.1.2.6.1: Summary table: Averse effects of 2-ethoxyethanol on the blood and hematopoietic system.

Table 4.1.2.6.1:

Summary table: Adverse effects of 2-ethoxyethanol on the blood and hematopoietic system

Exposure route, Species (male/female) **Dose Groups**	**Exposure duration**	**Adverse effects** **↑increase** **↓decrease**	**NOAEL** **NOAEC**	**Reference**
Inhalation (whole body) **rat** (m) 0, 370 ppm (1.37 mg/l)	7 hours/day 5 days/week 5 weeks	370 ppm (1.37 mg/l) ↑ splenic hemosiderosis ↓ myeloid cells in the spleen, fat replacement in the bone marrow, ↑ proportion of circulating immature granulocytes	-	Werner 1943
Inhalation (whole body) Sprague-Dawley CD **rat** (f) 0, 25, 100 or 400 ppm (equal to 0, 92.5, 390, or 1480 mg/m³)	6 hours/day 5 days/week 13 weeks	400 ppm (1480 mg/m^3) ↓ (minimal leucocyte counts)	400 ppm (1480 mg/m^3)	Barbee 1984, Bio/dynamics Inc 1983
Inhalation (whole body), New Zealand White **rabbit**	6 hours/day 5 days/week 13 weeks	400 ppm (1480 mg/m3) ↓ hematocrit, ↓ hemoglobin	100 ppm (390 mg/m³)	Barbee 1984, Bio/dynamics Inc 1983

(m/f) 0, 25, 100 or 400 ppm (equal to 0, 92.5, 390, or 1480 mg/m³)		concentration and ↓ erythrocyte count		
Inhalation (whole body) **dog (m/f)** 0, 840 ppm (3091 mg/m³)	7 hours/day 5 days/week 12 weeks	840 ppm (3091 mg/m^3) ↑ numbers of circulating immature granulocytes	-	Werner 1943
Oral by gavage Wistar **rat** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg) 93 or 186 mg/kg bw/day for 8 weeks, followed by 370 and 741 mg/kg bw/day respectively for 5 weeks	daily for 13 weeks	93 or 186 mg/kg bw/day for 8 weeks, followed by 370 and 741 mg/kg bw/day respectively for 5 weeks ↓ hemoglobin concentrations and ↓ packed cell volumes	93 mg/kg bw/day	Stenger 1971

Table 4.1.2.6.1 (contin.):

Summary table: Adverse effects of 2-ethoxyethanol on the blood and hematopoietic system

Exposure route, Species (male/female) **Dose Groups**	**Exposure duration**	**Adverse effects** **↑ increase** **↓ decrease**	**NOAEL** **NOAEC**	**Reference**
Oral	daily for	186 mg/kg bw/day↑	93 mg/kg	Stenger 1971

by gavage Wistar **rat** (m/f) 186 mg/kg bw/day for 8 weeks, followed by 743 mg/kg bw/day for 5 weeks	13 weeks	splenic hemosiderin	bw/day	
oral by gavage JCL-ICR **mouse** (m/f) 0, 500, 1000, 2000, 4000 mg/kg bw/day	5 days/week 5 weeks	2000 mg/kg bw/day ↓ white blood cell counts	1000 mg/kg bw/day	Nagano 1979
Oral by gavage Beagle **dog** (m/f) 0, 46, or 93 mg/kg bw/day	daily for 13 weeks	186 mg/kg bw/day ↓ hemoglobin level and ↓ hematocrit values	93 mg/kg bw/day	Stenger 1971
Oral in drinking water F344/N **rat** (m/f) 0, 1250, 2500, 5000, 10000, 20000 ppm (0, 109, 205, 400, 792, 2240 mg/kg for males; 0, 122, 247, 466, 804, 2061 mg/kg bw/d for females)	daily for 13 weeks	10000 ppm (792 mg/kg bw/day for males, 801 mg/kg bw/day for females) hemolytic anemia	5000 ppm (400 mg/kg bw/day for males, 466 mg/kg bw/day for females)	NTP 1993
Oral in drinking water B6C3F1 **mouse**	daily for 13 weeks	20000 ppm (5123 mg/kg bw/day for males and 7255 mg/kg bw/day for	5000 ppm (1304 mg/kg bw/day) for females	NTP 1993

(m/f) 0, 2500, 5000, 10000, 20000, 40000 ppm (0, 587, 971, 2003, 5123, 7284 mg/kg bw/day for males; 0, 722, 1304, 2725, 7255, 11172 mg/kg bw/day for females)		females) ↑ hematopoiesis in spleen		

Adverse effects on the male reproductive system

The effect of 2-ethoxyethanol on the male reproductive system has been intensively investigated. Degenerative changes in the germinal epithelium of the seminiferous tubules were consistently noted in the rat, mouse, rabbit and dog following exposure to 2-ethoxyethanol through the inhalation, the oral route, or by subcutaneous injection. These effects include testicular atrophy, degeneration of testicular tubules, germ maturation arrest and depletion of mature stages of germ cells, decrease in sperm counts and motility, and an increase in the number of abnormal sperm cells. Some further information is described within reproductive toxicity studies in section 4.1.2.9.

Inhalation experiments:

At 400 ppm (1480 mg/m³) 2-ethoxyethanol (6 hours/day, 5 days/week for 13-weeks), New Zealand white rabbits showed a decrease in testicular weights and slight focal seminiferous tubule degeneration in 3/10 animals. At the same concentration, no adverse effects on the testis of Sprague-Dawley CD rats were reported. The NOAEC for male reproductive effects in New Zealand white rabbits was 100 ppm, approx. 390 mg/m³ (Bio/dynamics Inc 1983; Barbee 1984). There was no inhalation study in rats which indicated testes degeneration.

Oral administration:

The lowest effect level of 2-ethoxyethanol which induces testes degeneration after repeated oral administration was 186 mg/kg bw/day in the rat and dog (13-week studies, Stenger 1971). Mice were less sensitive showing testes atrophy at doses from 1000 mg/kg bw/day (5-week study, Nagano 1979; 103-week study, Melnick, 1984). In general, the administration via drinking water was less toxic than the bolus application with via the oral gavage.

Summary of testicular effects in animals following repeated administration of 2-ethoxyethanol are presented in Table 4.1.2.6.2: Summary table: Adverse effects of 2-ethoxyethanol on the male reproductive system.

Table 4.1.2.6.2:

Summary table: Adverse effects of 2-ethoxyethanol on the male reproductive system

Exposure route **Species** **(male/female)** **Dose Groups**	**Exposure duration**	**Adverse effects** **↑ increase** **↓ decrease**	**NOAEL** **NOAEC**	**Reference**
Inhalation (whole body), Sprague-Dawley CD **rat** (m/f) 0, 25, 100 or 400 ppm (equal to 0, 92.5, 390, or 1480 mg/m³)	6 hours/day 5 days/week 13 weeks	-	400 ppm (1480 mg/m^3)	Barbee 1984, Bio/dynamics Inc 1983
Inhalation (whole body), New Zealand White **rabbit** (m/f) 0, 25, 100 or 400 ppm (equal to 0, 92.5, 390, or 1480 mg/m³)	6 hours/day 5 days/week 13 weeks	400 ppm (1480 mg/m^3) ↓ testicular weights, slight focal seminiferous tubule degeneration in 3/10	100 ppm (390 mg/m³)	Barbee 1984, Bio/dynamics Inc 1983
Oral by gavage Sprague-Dawley **rat** (m) 0, 250, 500, 1000 mg/kg bw/day, killed on day 2, 4, 7, or 11	daily for 11 days	500 mg/kg bw/day ↓ sperm count, changes in sperm motility, testicular degeneration seen in the later stages of primary spermatocyte development and secondary spermatocytes	250 mg/kg bw/day	Foster 1983, 1984
Oral	daily for	186 mg/kg bw/day testes: interstitial edema and	93 mg/kg bw/day	Stenger 1971

by gavage Wistar **rat** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg) 93 or 186 mg/kg bw/day for 8 weeks, followed by 370 and 741 mg/kg bw/day respectively for 5 weeks	13 weeks	maturation arrest of spermatogenesis		
Oral by gavage F344/N **rat** (m/f) 0, 500, 1000, 2000 mg/kg bw/day	5 days/week 17-18 weeks	2000 mg/kg bw/day testicular atrophy 2000 mg/kg bw/day: terminated at week 17/18 due to high rates of mortalities due to stomach ulcers, testes atrophy	-	Melnick 1984
Oral by gavage F344/N **rat** (m/f) 0, 500, 1000, 2000 mg/kg bw/day	5 days/week 103 weeks	-	1000 mg/kg bw/day	Melnick 1984

Table 4.1.2.6.2 (contin.):

Summary table: Adverse effects of 2-ethoxyethanol on the male reproductive system

Exposure route, Species **(male/female)** **Dose Groups**	**Exposure duration**	**Adverse effects** **↑ increase** **↓ decrease**	**NOAEL** **NOAEC**	**Reference**

Oral by gavage ICL-ICR **mouse** (m/f) 0, 500, 1000, 2000, 4000 mg/kg bw/day	5 days/week 5 weeks	≥ 1000 mg/kg bw/day ↓ testes weight, testicular atrophy, tubular degenerative hypospermia	500 mg/kg bw/day	Nagano 1979
Oral by gavage B6C3F1 **mouse** (m/f) 0, 500, 1000, 2000 mg/kg bw/day	5 days/week 17-18 weeks	2000 mg/kg bw/day ↓ size of testes testicular atrophy 2000 mg/kg bw/day terminated at week 17/18 due to high rates of mortalities due to stomach ulcers (m)	-	Melnick 1984
Oral by gavage B6C3F1 **mouse** (m/f) 0, 500, 1000, 2000 mg/kg bw/day	5 days/week 103 weeks	≥ 1000 mg/kg bw/day testicular atrophy	500 mg/kg bw/day	Melnick 1984
Oral by gavage Beagle **dog** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg)	daily for 13 weeks	186 mg/kg bw/day degenerative changes in testes in 3/3	93 mg/kg bw/day	Stenger 1971
Oral in feed Wistar **rat** (m/f) 0, 0.01, 0.05, 0.25, 1.25% (0. 8, 39, 190,	daily for 90 days	-	1000 mg/kg bw/day	Union Carbide 1962

1007 mg/kg bw/day for males; 0, 9, 45, 222, 1039 mg/kg bw/day for females)				
Oral in feed **rat** (m/f) 1.45% (900 mg/kg bw/day)	daily for 2 years	1.45% (900 mg/kg bw/day) enlargement of testes, tubular atrophy, interstitial edema	-	Morris 1942

Table 4.1.2.6.2 (contin.):

Summary table: Adverse effects of 2-ethoxyethanol on the male reproductive system

Exposure route, Species **(male/female)** **Dose Groups**	**Exposure duration**	**Adverse effects** **↑ increase** **↓ decrease**	**NOAEL** **NOAEC**	**Reference**
Oral in drinking water F344/N **rat** (m/f) 0, 300, 600, 900, 1500, or 2500 mg/kg bw/day	daily for 2 weeks	1500 mg/kg bw/day degeneration of the seminiferous tubules	300 mg/kg bw/day	NTP 1993
Oral in drinking water F344/N **rat** (m/f) 5000, 10000, or 20000 ppm (407, 792, 2390 mg/kg bw/day)	daily for 60 days	10000 ppm (792 mg/kg bw/day) degeneration of the seminiferous tubules	-	NTP 1993
Oral in drinking water F344/N **rat** (m/f)	daily for 13 weeks	≥ 2500 ppm (≥ 205 mg/kg bw/day) abnormal sperm morphology, hypospermia	1250 ppm (109 mg/kg bw/day)	NTP 1993

0, 1250, 2500, 5000, 10000, 20000 ppm (0, 109, 205, 400, 792, 2240 mg/kg for males; 0, 122, 247, 466, 804, 2061 mg/kg bw/d for females)		5000 ppm (400 mg/kg bw/day) ↓ epididymal weights, testicular degeneration ≥ 10000 (≥ 792 mg/kg bw/day) ↓ testis size, ↓ absolute and relative testis, and ↓ epididymal weights, testicular degeneration		
Oral in drinking water B6C3F1 **mouse** (m/f) 0, 2500, 5000, 10000, 20000, 40000 ppm (0, 587, 971, 2003, 5123, 7284 mg/kg bw/day for males; 0, 722, 1304, 2725, 7255, 11172 mg/kg bw/day for females)	daily for 13 weeks	≥ 20000 ppm (5123 mg/kg bw/day) ↓ testicular weight, abnormal spermmorphology, hypospermia 40000 ppm (7284 mg/kg bw/day) testicular atrophy, degeneration of the germinal epithelium in seminiferous tubules,	10000 ppm (2003 mg/kg bw/day)	NTP 1993
Subcutaneous application Wistar **rat** (m/f) 0, 100, 200, 400, or 800 µl/kg bw/day (approx. 0, 93, 186, 372, or 744 mg/kg bw/day)	daily for 4 weeks	372 mg/kg bw/day maturation arrest of spermatogenesis, interstitial edema and polynuclear cell infiltration	186 mg/kg bw/day	Stenger 1971

Effects on the kidneys

2-ethoxyethanol does not appear to cause significant nephrotoxicity after repeated-dose administration.

Inhalation experiments:

No effects on kidneys were seen after inhalation exposure to rats, rabbits, or dogs (Werner 1943; Goldberg 1964; Bio/dynamics Inc. 1983; Barbee 1984).

Oral administration:

Distension and flattening of the distal and convoluted tubules was observed in 50% of dogs dosed orally with 186 mg/kg bw/day 2-ethoxyethanol for 13 weeks (Stenger 1971).

Dietary administration of approximately 900 mg/kg bw/day 2-ethoxyethanol to rats for 2 years produced in a few animals tubular atrophy and focal fibrosis (Morris 1942).

Subcutaneous administration:

Subcutaneous administration of 2-ethoxyethanol to rats for 4 weeks at dosages of $\geq$ 370 mg/kg bw/day resulted in swelling of tubular epithelium and constriction of the lumen, particularly in the convoluted tubules (Stenger 1971).

Summary of effects on kidneys in experimental animals following repeated administration of 2-ethoxyethanol are presented in Table 4.1.2.6.3: Summary table: Adverse effects of 2-ethoxyethanol on kidneys.

Table 4.1.2.6.3:

Summary table: Adverse effects of 2-ethoxyethanol on kidneys

Exposure route, Species **(male/female)** **Dose Groups**	**Exposure duration**	**Adverse effects** **↑ increase** **↓ decrease**	**NOAEL**	**Reference**
Oral by gavage Beagle **dog** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg)	daily for 13 weeks	186 mg/kg bw/day distension and flattening of the distal and convoluted tubules in 50% of dogs	93 mg/kg bw/day	Stenger 1971
Oral in feed **rat** (m/f) 1.45% (approx. 900 mg/kg bw/day)	daily for 2 years	1.45% (approx. 900 mg/kg bw/day) tubular atrophy, focal fibrosis	-	Morris 1942
Subcutaneous application Wistar **rat** (m/f) 0, 100, 200, 400, or 800 µl/kg bw/day (approx. 0, 93, 186, 372, or 744 mg/kg bw/day)	daily for 4 weeks	2372 mg/kg bw/day swelling of tubular epithelium, constriction of the lumen, particularly of the convoluted tubules	186 mg/kg bw/day	Stenger 1971

Behavioural and neurological effects

Although neurological dysfunction has been noted as a consequence of exposure to glycol ethers in man, there is very little consistent evidence for an effect on the function of the nervous system in experimental animals.

No inhibition of conditioned responses was noted in tests performed on rats exposed to 4000 ppm (14960 mg/m^3) 2-ethoxyethanol 4 hours/day, 5 days/week for two weeks (Goldberg 1964). Somnolence and slight ataxia occurred after subcutaneous administration to rats at dosage of $\geq$372 mg/kg bw/day 2-ethoxyethanol for 4 weeks, and after intravenous injection of 465 mg/kg bw/day to dogs.

Summary on behavioural and neurological effects to 2-ethoxyethanol

There have been a few reports on adverse effects of 2-ethoxyethanol on the function of the nervous system and altered behavioural effects in animals. Based on the data presented, there were no specific indications on neurotoxicity. However, there are insufficient data of allow appraisal of the effects of 2-ethoxyethanol on the nervous system. Furthermore, microscopic examination of the nervous system was often not carried out.

Effects on the liver

Effects on the liver have been inconsistently seen in repeated-dose toxicity studies with alkoxyethanols. The effects reported include reduced cytoplasmatic density, cloudy swelling, disruption of lobular structure, elevated plasma fibrinogen concentration, reduced serum protein levels and elevated liver weight. These effects, many of which are reversible, have occurred after exposure to levels of approx. 300 ppm 2-ethoxyethanol. Most effects obtained in the liver was frequently an increased weight (in absence of pathological change) following high doses of 2-ethoxyethanol (Stenger 1971; Werner 1943).

Summary on liver effects to 2-ethoxyethanol

Apart from organ toxicity, the liver has frequently shown an increased weight (in absence of pathological change) following high doses of 2-ethoxyethanol. Hepatic effects are thus not a major consequence of 2-ethoxyethanol administration.

From numerous animal studies by repeated inhalation, oral, and subcutaneous administration of 2-ethoxyethanol, the NOAEL/NOAEC for main effects of 2-ethoxyethanol was derived as shown in the following table, Table 4.1.2.6.4: Summary table: NOAEL/NOAEC values for 2-ethoxyethanol for all adverse effects from animal studies.

Table 4.1.2.6.4:

Summary table: NOAEL/NOAEC values for 2-ethoxyethanol for all adverse effects from animal studies

Exposure route **Species** **(male/female)**	**Exposure duration**	**NOAEL** **NOAEC** **(effect)**	**Reference**
Inhalation (whole body), CFE **rat** (f) 0, 500, 1000, 2000, 4000 ppm (0, 1870, 3740, 7480, 14960 mg/m³)	4 hours/day, 5 days/week 2 weeks	500-4000 ppm as vapour (no effects in behaviour)	Goldberg 1964
Inhalation (whole body), Sprague-Dawley CD **rat** (m/f) 0, 25, 100, 400 ppm (0, 92.5, 390, 1480 mg/m³)	6 hours/day, 5 days/week, 13 weeks	400 ppm (1480 mg/m³) (no biological significant effects on the blood and hematopoietic system, no male reproductive effects)	Barbee 1984, Bio/dynamics Inc. 1983
Inhalation	6 hours/day	100 ppm (390 mg/m³)	Bio/dynamics Inc 1983, Barbee 1984

(whole body) New Zealand White rabbit (m/f) 0, 25, 100, 400 ppm (0, 92.5, 390, 1480 mg/m³)	5 day/week 13 weeks	(no male reproductive effects no effects on the blood and haematopoietic system)	

Oral by gavage Sprague-Dawley CD **rat** (m) 0, 250, 500, 1000 mg/kg bw/day, killed on day 2, 4, 7, or 11	daily for 11 days	250 mg/kg bw/day (no male reproductive effects)	Foster 1983, 1984
Oral by gavage Wistar **rat** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg) 93 or 186 mg/kg bw/day for 8 weeks, followed by 370 and 741 mg/kg bw/day respectively for 5 weeks	daily for 13 weeks	93 mg/kg bw/day (no effects on the blood and hematopoietic system, no male reproductive effects)	Stenger 1971
Oral in feed	daily for 90 days	200 mg/kg bw/day (no biological significant effects)	Union Carbide 1962

Wistar **rat** (m/f) 0, 0.01, 0.05, 0.25, 1.25% (0, 8, 39, 190, 1007 mg/kg bw/day for males; 0, 9, 45, 222, 1039 mg/kg bw/day for females)			
Oral in drinking water F344/N **rat** (m) 0, 1250, 2500, 5000, 10000, 20000 ppm (0, 109, 205, 400, 792, 2240 mg/kg for males)	daily for 13 weeks	1250 ppm (109 mg/kg bw/day) (no effects on relative thymus weights)	NTP 1993
Oral in drinking water F344/N **rat** (f) 0, 1250, 2500, 5000, 10000, 20000 ppm (0, 122, 247, 466, 804, 2061 mg/kg bw/d for females)	daily for 13 weeks	5000 ppm (466 mg/kg bw/day) (no effects on the blood and hematopoietic system, no effects on examined organs and tissues)	NTP 1993

Table 4.1.2.6.4 (contin.):

Summary table: NOAEL/NOAEC values for 2-ethoxyethanol for all adverse effects from animal studies

Exposure	**Exposure**	**NOAEL**	**Reference**

route **Species** **(male/female)** **Dose Groups**	**duration**	**NOAEC** **(effect)**	
Oral by gavage JCL-ICR **mouse** (m/f) 0, 500, 1000, 2000, 4000 mg/kg bw/d	5 days/week 5 weeks	500 mg/kg bw/day (no effects on the blood, no effects on the male reproductive system)	Nagano 1979
Oral in drinking water B6C3F1 **mouse** (m) 0, 2500, 5000, 10000, 20000, 40000 ppm (0, 587, 971, 2003, 5123, 7284 mg/kg bw/day for males)	daily for 13 weeks	20000 ppm (5123 mg/kg bw/day) (no male reproductive effects, no ↑ hematopoiesis in the spleen)	NTP 1993
Oral in drinking water B6C3F1 **mouse** (f) 0, 2500, 5000, 10000, 20000, 40000 ppm (0, 722, 1304, 2725, 7255, 11172 mg/kg bw/day for females)	daily for 13 weeks	5000 ppm (1304 mg/kg bw/day) (adrenal gland hypertrophy, no ↑ hematopoiesis in the spleen)	NTP 1993

Oral by gelatine capsule Beagle **dog** (m/f) 0, 50, 100, 200 µl/kg bw/day (0, 46, 93, 186 mg/kg)	daily for 13 weeks	93 mg/kg bw/day (no effects on the blood and hematopoietic system, no male reproductive effects)	Stenger 1971
Subcutaneous application Wistar rat (m) 0, 100, 200, 400, or 800 µl/kg bw/day, approx. 0, 93, 186, 372, or 744 mg/kg bw/day	daily for 4 weeks	186 mg/kg bw/day (no male reproductive effects)	Stenger 1971

Summary of effects of repeated exposure in experimental animals:

In experimental animals, the most prominent adverse effects related to repeated exposures to 2-ethoxyethanol were evident in the haematopoietic system in both genders and in the male reproductive organs. Besides, adverse effects in a number of other organs (kidneys: tubular degeneration, adrenal gland hypertrophy, thymus atrophy, liver cell degeneration) were seen, but there were considered of lower significance since the dosages where they occurred were relatively high, their occurrence was less consistent across studies or changes were not severely graded.

Adverse effects on the haematopoietic system

Mild haemolytic anaemia and corresponding indirect effects such as increased hemosiderin deposition in the spleen and intensified extramedullary haematopoiesis were observed in a number of studies that included at least a basic set of haematology parameters. The lowest effective dose was 186 mg/kg bw/day for the oral route (Stenger et al., 1971, 13-week study, rat and dog) and 400 ppm (1480 mg/m³) for the inhalation route (13-week study, rat) (Barbee et al.,1984, Bio/dynamics Inc., 1983). Marked anaemia was observed at dosages of 10000 ppm in drinking water (about 800 mg/kg bw/day) (NTP, 1993).

Other effects included transient leucopoenia during the first weeks of treatment (NTP, 1993), and a reduction of myeloid cells in the spleen (Werner, 1943) that along with thymus atrophy (NTP, 1993, Ma-Hock et al., 2005) might indicate an immunosuppressive potential. However, its evidence is weak due to lack of consistency among studies. Leucocytosis and the shift to immature granulocytes could be caused by degenerative-inflammatory lesions in organs (most likely the testes effects in the 13-week study, NTP, 1993).

Adverse effects on the blood and haematopoietic system occurred at the same 2-ethoxyethanol concentrations than adverse effects on the male reproductive system.

Adverse effects on the male reproductive system

The effects of 2-ethoxyethanol on the male reproductive system have been intensively investigated. Degenerative changes in the germinal epithelium of the seminiferous tubules were consistently notedin the rat, mouse, rabbit and dog following exposure to 2-ethoxyethanol through the inhalation, oral route or by subcutaneous injection. These effects include testicular atrophy, degeneration of testicular tubules, germ maturation arrest and depletion of mature stages of germ cells, decrease in sperm counts and motility, and an increase in the number of abnormal sperm cells.

The lowest effective dose (LOAEL) where testes toxicity occurred was 186 mg/kg bw/day estimated in a 13-week rat study (Stenger et al., 1971). Much higher dosages were needed when 2-ethoxyethanol was administered by feed. Via inhalation, the lowest effective concentration was 400 ppm (1480 mg/m³) in rabbits (Barbee et al., 1984, Bio/Dynamics Inc., 1983).

No/Lowest-observed-effect levels/concentrations

Inhalation

The NOAEC of 100 ppm (390 mg/m³) is the lowest NOAEC based on male reproductive effects in rabbits for exposure and uptake of 2-ethoxyethanol via inhalation (Bio/dynamics Inc 1983, Barbee 1984). This was derived from a 13-week study which was well performed and the results were in conformity with the findings of the other studies.

13-week inhalation/ New Zealand white rabbit **$NOAEC_{sys}$ 100 ppm (390 mg/m³)**

Based on the data available a NOAEC for local effects on the respiratory tract could not be derived.

Oral administration

The lowest oral NOAEL for effects on the blood and hematopoietic system, and male reproductive system in rats was established at 93 mg/kg bw/day (Stenger 1971). This was derived from a 13-week (gavage) study which was not conducted according OECD/EEC guidelines for oral repeated dose studies on rodents. However, the result was in conformity with findings of other studies. In 13-week drinking water study on F344/N rats (method similar to OECD TG 408) adverse effects on the male reproductive system were observed at ≥2500 ppm, equivalent to ≥205 mg/kg bw/day (NTP, 1993). The NOAEL for male reproductive effects was estimated at 1250 ppm (equivalent to 109 mg/kg bw/day).

13-week oral (gavage)/Wistar rat **NOAEL 93 mg/kg bw/day**

Human data:

There are no data on chronic toxic effects reported in the literature. In workers (painters) in ship industry anemia and leukopenia have been described; however these persons were exposed to mixtures with other solvents and heavy metals (Welch 1988).

Conclusion: Since none of the adverse effects observed occurred in the dose-ranges critical for R48 classification, no classification is required for repeated toxicity for the oral and the inhalation route.

4.1.2.7 Mutagenicity

All mutagenicity tests in vitro and with animals were conducted with 2-ethoxyethanol of high purity.

Bacterial in vitro assays

In bacterial gene mutation tests 2-ethoxyethanol was negative with and without S-9 mix in S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 up to approximately 100'000 µg/plate (Union Carbide 1983; Shimizu et al. 1985; Zeiger et al. 1985).

A test with E. coli WP2uvr was also negative with and without S-9 mix up to doses of 5000 µg/plate (Shimizu et al. 1985).

In vitro assays with mammalian cells

A mammalian cell gene mutation assay with 2-ethoxyethanol using CHO cells (HPRT locus) was negative with S-9 mix up to doses of 36 000 µg/ml (400 mmol/l) and without S-9 mix up to doses of 32 000 µg/ml (355 mmol/l); there were no cytotoxic effects (Union Carbide 1984b).

In the absence of S-9 mix, two chromosomal aberration assays with CHO cells were positive in a dose-dependent manner for doses ranging from 583 µg/ml (64 mmol/l) to 9510 µg/ml (105 mmol/l) in one test and from 11 600 µg/ml (129 mmol/l) to 20 000 µg/ml (222 mmol/l) in the other test. In the highest tested concentration of 20 000 µg/ml (222 mmol/l) 25.0% aberrant cells were induced as compared to 3.0% in the control culture; a concentration of 4780 µg/ml (53 mmol/l) was negative. With S-9 mix concentrations ranging from 4780 to 26 000 µg/ml (289 mmol/l) were negative. No information about cytotoxic effects aswas given (Galloway et al. 1987; Union Carbide 1984c).

2-ethoxyethanol was analysed in tthree SCE tests with CHO cells with and without S-9 mix (Union Carbide 1984a; Union Carbide 1984b; Galloway et al. 1987). With S-9 mix a clearly positive finding was obtained in one test (up to 19.1 SCE per cell as compared to 8.9 in the control) in a dose range from 20 000 µg/ml (222 mmol/l) up to 35 000 µg/ml (388 mmol/l). Two further tests with S-9 mix led to weak increases of SCE frequencies. Without S-9 mix in two tests the results were clearly positive (up to 28.2 SCE per cell as compared to 8.0 in the control) in dose ranges from 3170 µg/ml (35 mmol/l) up to 9510 µg/ml (105 mmol/l) and from 10 000 µg/ml (111 mmol/l) up to 20 000 µg/ml (222 mmol/l). One further test led to a weak increase of SCE frequencies. In the Galloway study, negative findings were obtained for a concentration of 951 µg/ml (10.5 mmol/l) with and without S-9 mix Galloway et al. 1987); in the Union Carbide studies only extremely high concentrations leading to positive effects were used (Union Carbide 1984a; 1984b).

Assays with Drosophila melanogaster

In general, 2-ethoxyethanol was negative in sex-linked recessive lethal tests in Drosophila melanogaster. No genetic effects were found after oral feeding (concentrations of 5100 ppm and 20'000 ppm in feeding solution over a period of 3 days) and after injection of a test solution with a concentration of 5200 ppm (Mason et al. 1992; Valencia et al. 1985). After injection of a test solution with a concentration of 50'000 ppm there was an equivocal result (Valencia et al. 1985). There were lethal effects in all tests.

In vivo assay with mammals

An in vivo micronucleus test with 2-ethoxyethanol was negative in bone marrow cells of mice after intraperitoneally injection of doses up to 2125 mg/kg. The highest tested dose induced marginal cytotoxic effects; information on lethal effects or clinical symptoms were not given (Union Carbide 1985)

Human study

A genotoxicity study on workers exposed to glycolethers was negative (Angerer et al. 1991). The study was performed with peripheral lymphocyte cultures retrospectively; effects of glycolethers in an exposed group were compared to a non-exposed group. As a result of the personal air monitoring it was shown that the mean concentration of each of the glycolethers was less than 10% of the corresponding MAK-value (maximum concentration at the work place). There were no effects of glycolethers both on micronucleus frequencies (7.1 micronuclei per 500 binucleated cells in the exposed group as compared to 7.7 micronuclei per 500 binucleated cells in the control group) as well as on SCE frequencies (mean rate of 11.2 SCE in exposed and in non-exposed group).

Conclusion

In conclusion, 2-ethoxyethanol was negative in bacterial gene mutation tests and in a gene mutation test with mammalian cells. In vitro chromosomal aberration tests (without S-9 mix) and in vitro SCE tests (with and without S-9 mix) were positive at extremely high concentrations (35 mmol/l and higher). Due to these tests the substance seems to have a marginal mutagenic potential for mammalian cells in culture. The negative in vivo micronucleus test indicates that this potential is unlikely to be expressed in vivo.

4.1.2.8 Carcinogenicity

Animal data:

Inhalation experiment

No carcinogenicity or chronic toxicity studies were available using the inhalation route of exposure.

Oral administration:

Gavage studies (rat and mouse)

Groups of 50 Fischer 344/N rats and 50 B6C3F1 mice of both sexes were administered 2-ethoxyethanol in deionized water by gavage five times per week for 103 consecutive weeks at dose levels of 0, 500, 1000, and 2000 mg/kg bw. This followed by a one-week observation period. Data on hematology and/or clinical chemistry were not available. Because mortality was high in the 2000 mg/kg bw dose groups, survivors were sacrificed at week 17 to 18. Two types of gross lesions were commonly seen in the 2-year study to 2-ethoxyethanol. First, testicular atrophy was observed in male rats that died early in this study and in the medium- and high-dose male mouse groups. Second, stomach ulcers were observed in many of the high-dose male and female rats. Early mortality in the high-dose groups of both sexes appeared to be due to stomach ulcers.

In the study with rats and mice, no increase in the incidence of tumor rates and no significant differences in the total tumor incidences between the 2-ethoxyethanol-treated and control groups were reported (Melnick 1984). Since the conclusion was drawn on macroscopic findings only, the reliability of the outcome is limited by the lack of histopathology data.

Diet study (rat)

In a chronic diet study, male and female rats (20 animals/group) received 1.45% (equivalent to approx. 900 mg/kg bw/day) 2-ethoxyethanol administered in the diet for 2 years. No data on hematology and clinical chemistry parameters were available. Histopathology from selected tissues and organs was reported. About half the animals were examined microscopically. Of these two-thirds of the males examined showed marked testicular enlargement, interstitial oedema and atrophy of the seminiferous tubules. In a few rats slight lesions on kidneys were reported. These consisted of tubular atrophy with less marked glomerular atrophy; tubular casts, usually hyaline, and slight degrees of lymphocytic infiltration and fibrosis.

Tumour rates of distinct tumour types did not increase in the treated groups compared to those of the control groups. NNoincrease in the incidence of tumour rates was observed and no significant differences in the total tumor incidences between the 2-ethoxyethanol-treated and control groups were noted. Therefore this 2-year feeding study on rats gave no indication on a carcinogenic potential of 2-ethoxyethanol at a dose level of 900 mg/kg bw/day (Morris 1942).

Dermal application:

No carcinogenicity or chronic toxicity studies were available using the dermal route of exposure.

Human data:

No data are available.

Summary and discussion of carcinogenicity:

No carcinogenicity studies with compliance to the today's testing guidelines are available.

The findings of the two long-term studies of limited quality give no hind that 2-ethoxyethanol has a carcinogenic potential in experimental animals.

Conclusion: There are no adequate data to decide on the carcinogenic potential of 2-ethoxyethanol. Since there is no concern from other data, no classification is proposed at the moment.

4.1.2.9 Toxicity for reproduction

Animal data:

Fertility impairment

There is one study available, designated as fertility assessment by continuous breeding (Lamb et al. 1985), where groups of 20 male and 20 female CD-1 mice were exposed to 2-ethoxyethanol (99.4% purity) via drinking water at concentration levels of 0.5, 1.0, and 2.0% resulting in an intake of approximately 800, 1500, and 2600 mg/kg bw/day. Animals were continuously exposed over a premating period of 7 days followed by a breeding period during which they were randomly paired (one male: one female) and cohabited for 14 weeks. Animals from the 2.0% dose group as well of the 1.0% dose group were also tested in a cross over mating trial (treated females cohabited with control males and vice versa) to determine whether the males and females or both sexes had comprised reproductive performance when matched with control animals. Investigations on the reproductive performance of the offspring had not been performed in this study.

Daily water consumption was reduced in the 2.0% dose group but without any significant loss in body weight. No litters at all were found when males and females received 2.0% 2-ethoxyethanol in the drinking water. Also in the 1.0% dose group two of the 20 pairs did not deliver any litters during this study, whereas all pairs in the control and in the 0.5% dose group had at least one litter. At the 1.0% dose level there was a decrease in the mean number of litters, also the number of live pups per litter was reduced and the proportion of pups born alive, and the mean live pup weight were also significantly reduced when compared to controls. The animals in the 0.5% dose group did not seem to be adversely affected with respect to these endpoints.

The cross over mating trial for the 2.0% dose group revealed that treated females had no fertile mating at all, while treated males had significantly fewer fertile mating than the control pairs and a slightly decreased number of live pups. In the cross over mating trial of the 1.0% dose group there was a decrease, though not statistically significant, in the percent fertile matins for both the treated males cohabited with control females and the treated females cohabited with control males when compared to the control pairs. Also the number of live pups per litter was slightly lower in the treated female group; the pup weight also seemed to

be decreased in that treated group. Since there were significant effects on fertility and reproduction in both treated males and females, all animals had been necropsied and reproductive tract and gonadal tissues were weighed and examined for gross and histological effects. A profound dose related decrease in sperm motility and an increase in the percentage of morphologically abnormal sperm were revealed. Cauda epididymis weight and cauda epididymis sperm counts were also reduced. Treatment-related lesions were identified in the testis, including decreased testis weight and decreased spermatogenesis. The dose-related decrease in spermatogenesis conformed with findings of testicular atrophy. No gross or microscopic lesions were significantly increased in the female mice.

The results from this study indicate that 2-ethoxyethanol causes a profound effect on the reproductive function in CD-1 mice of both sexes at the 1.0% and 2.0% dose level and a no observed adverse effect level (NOAEL) for both sexes of about 0.5% (according to approximately 800 mg/kg bw/day) when applied via drinking water.

In a further study groups of 29 to 38 females rats were exposed to 2-ethoxyethanol for three weeks (pregestational exposure) at concentrations of 0 ppm, 150 $\pm$ 18 ppm or 649 $\pm$ 50 ppm, 7 hr/day, 5 days/week and/or 0 ppm, 202 $\pm$ 11 ppm or 767 $\pm$ 22 ppm on g.d. 1-19 , 7 hr/day (gestational exposure). The animals were terminated on g.d. 21 and fetuses examined for external, visceral and skeletal defects. Three weeks of exposure of nonpregnant rats to either pregestational exposure level did not appear to alter food consumption or body weight. Gestational exposure to the higher level lead to some reduced weight gain in the late treatment period (g.d. 17 and 21) only and to a decrease in mean relative dam liver weights. It was reported that exposure to 2-ethoxyethanol did not appear to alter mating behaviour, breeding performance, or fertility as indicated by percentage of pregnant dams at sacrifice (Andrew and Hardin 1984, Andrew et al. 1981, c.f. developmental toxicity).

Furthermore, data are available from several investigations on the effects of 2-ethoxyethanol on the male reproductive system and from repeated dose toxicity studies (>weeks), which have been described in more detail already in section 4.1.2.6 and the essential results of which are compiled in Table 4.1.2.9.

Table 4.1.2.9 Compilation of data of effects of 2-ethoxyethanol on the male reproductive system

Species	Protocol	Results
Inhalatory administration		
Rat (Alpk/Ap)	4 500 ppm 3 h, single exposure	testes weight ↓, testicular atrophy, hematuria (Doe 1984a)

Species	Protocol	Results
Rat (Sprague-Dawley)	25, 100, 400 ppm (6 h/day, 5 d/week) whole body 13 weeks	400 ppm: NOEL (Barbee et al. 1984; Bio/dynamics Inc 1983)
Rabbit (New Zealander)	25, 100, 400 ppm (6 h/day, 5 d/week) whole body 13 weeks	400 ppm: body weight ↓, testes weight ↓, slight degeneration of seminal tubuli 100 ppm: NOEL (Barbee et al. 1984; Bio/dynamics Inc 1983)
Oral administration		
Rat (albino, inbred strain)	1.45 % in diet (~ 900 mg/kg/d) 2 years	testicular edema, atrophy of germinal epithelium (Morris et al. 1942)
Rat (F334/N)	1250, 2500, 5000, 10000, 20000 ppm in drinking water, 13 weeks	> 10000 ppm: testicular size ↓, abs. + rel. testes weight ↓ > 5000 ppm: testicular degeneration > 2500 ppm: sperm count significantly ↓ 1250 ppm: NOAEL for male reproductive effects (109 mg/kg bw/d) (NTP 1993)
Rat (F 344/N)	500, 1000 mg/kg/d, 2 years (gavage) 2000 mg/kg/d, 17 - 18 weeks (gavage)	enlarged testis with or without evidence of a mass testicular size ↓, testicular atrophy (Melnick 1984)

Species	Protocol	Results
Rat (Sprague-Dawley)	250, 500 and 1 000 mg/kg/d (gavage) 11 days	500, 1000 mg/kg/d: histopathological testicular changes (spermatocyte degeneration) 250 mg/kg/d: NOEL (Foster et al. 1983, 1984)
Rat (Long-Evans)	150 and 300 mg/kg/d (gavage, 5d/week) 6 weeks	300 mg/kg/d: testes weight ↓, spermatid count ↓, epididymal sperm count ↓, % normal sperm morphology ↓ 150 mg/kg/d: in mated groups epididymal sperm count ↓, % normal sperm morphology ↓ (Hurtt and Zenick 1986)
Rat (Long-Evans)	936, 1872 and 2808 mg/kg/d (gavage) 5 days postobservation period: 16 weeks 936 mg/kg/d (gavage, 5d/week) 6 weeks	1872, 2808 mg/kg/d: rapid decline in sperm count, azoospermia, resp. severe oligozoospermia by week 7 936 mg/kg/d: by weeks 7 sperm count ↓, abnormal sperm morphology ↑ partial or complete recovery of the effects by week 14 to 16, no treatment related effects on copulatory behaviour (Oudiz et al. 1984; Zenick et al. 1984) testes, epididymides and cauda epididymides weights ↓, sperm count and -motility ↓, % normal sperm morphology ↓ at week 5 and 6 no treatment related effects on copulatory behaviour (Oudiz and Zenick 1986; Zenick et al. 1984)
Rat (Wistar)	50, 100, 200, 100/400, 200/800 µL/kg bw/day 13 weeks (7d/week)	200, 200/800 µL/kg bw/day: testicular edema, absence of more mature sperm cells 100 µL/kg bw/day: NOEL (= 93 mg/kg/d) (Stenger et al. 1971)

Species	Protocol	Results
Mouse (B6C3F1)	2500, 5000, 10000, 20000, 40000 ppm in drinking water, 13 weeks	40000 ppm: abs. + rel. testes weight ↓, histopathol. degeneration of testes 20000 ppm: sperm count and motility significantly ↓ 10000 ppm: NOEL (= 2003 mg/kg/d) (NTP 1993)
Mouse (B6C3F1)	500, 1000 mg/kg/d 2 years (5d/week) (gavage) 2000 mg/kg/d 17 - 18 weeks (5d/week) (gavage)	testicular size ↓ testicular size ↓, testicular atrophy (Melnick 1984)
Mouse (ICL-ICR)	500, 1 000, 2 000 and 4 000 mg/kg/d 5 weeks (5 d/week)	4 000 mg/kg/d: lethal 2 000 mg/kg/d: testes weight ↓, leucopenia 1 000 mg/kg/d: testes weight ↓ marked testicular atrophy 500 mg/kg/d: NOEL (Nagano et al. 1979)
Dog (Beagle)	50, 100, 200 µL/kg bw/day 13 weeks (7d/week)	200 µL/kg bw/day: testicular edema, absence of more mature sperm cells 100 µL/kg bw/day: NOEL (= 93 mg/kg/d) (Stenger et al. 1971)
Subcutaneous administration		

Species	Protocol	Results
Rat (Wistar)	100, 200, 400 and 800 µL/kg bw/day 4 weeks (7d/week)	400 and 800 µL/kg bw/day: testicular edema, absence of more mature sperm cells 200 µL/kg bw/day: NOEL (= 186 mg/kg/d) (Stenger et al. 1971)

Developmental toxicity:

Developmental toxicity of 2-ethoxyethanol was investigated in several studies with various routes of exposure on various species.

Inhalatory route of exposure

In a study on Alpk/AP rats and Dutch rabbits (Doe 1984b; Tinston et al. 1983a, 1983b) pregnant females were exposed to 2-ethoxyethanol vapours by whole chamber administration.

In the study with rats 24 females/group were exposed to 2-ethoxyethanol at concentrations of 0, 10, 50, or 250 ppm, 6h/day on g.d. 6-15. The animals were terminated on g.d. 21 and fetuses of finally 21 to 24 litters were examined for external, visceral and skeletal defects. There was no evidence for any maternal toxicity at 10 and 50 ppm, whereas at 250 ppm some slight yet statistically significant hematological changes were observed. There was a higher level of preimplantation loss in all exposed groups compared with controls, although this was statistically significant only in the 10 and 50 ppm groups. At 250 ppm there was a marked increase in the incidence of late uterine deaths and in the proportion of dams affected, indicating an increased postimplantation loss. Also mean fetal body weight was statistically significantly reduced at 250 ppm. There were no major skeletal defects identified in the offspring in this study, but overall there was a fetotoxic effect at 250 ppm, indicated by reduced ossification, which could be related to the retarded fetal growth observed at this level. Also an increased incidence of skeletal variants in the 250 ppm group was consistent with a fetotoxic effect. A small number of these changes was observed also at 50 ppm (i.e. unossified cervical centra, partial ossification of the second sternebrae, extra ribs).

In the study with rabbits 24 females/group were exposed to 2-ethoxyethanol at concentrations of 0, 10, 50, or 175 ppm, 6h/day on g.d. 6-18. The animals were terminated on g.d. 29 and fetuses of finally 16 to 22 litters were examined for external, visceral and skeletal defects. There were no effects observed in the does on body weight gain or food consumption nor any clinical abnormalities which could be attributed to exposure to 2-ethoxyethanol. Also, there was no evidence for embryotoxicity or fetotoxicity from the litter data, since fetal weights, numbers of fetuses and the incidence of intrauterine deaths in the groups exposed to 2-ethoxyethanol were similar to the controls. However, although there was no statistically significant increase in the incidence of fetal external or visceral defects in any of the exposure levels, in the 175 ppm group there was one fetus with a cardiovascular defect and one other with an abdominal wall defect. Also the incidence of skeletal defects (increased incidence of presacral vertebrae, retarded ossification) and of skeletal variants (mainly extra ribs of both

short and normal length) was statistically significantly greater in the 175 ppm than in the control group. The incidence of skeletal variants was also slightly yet not statistically significantly increased in the 10 and 50 ppm groups. The authors summarized that the overall results of their study in rats and rabbits indicate that levels of 175 to 250 ppm may be around the threshold level for teratogenicity. 175 and 250 ppm were shown to be fetotoxic in both species, and 50 ppm were shown to be mildly fetotoxic in rats. It was concluded that 10 ppm was a clear no-effect level in both species.

In a further inhalation study female Wistar rats as well as New Zealand White rabbits (Andrew and Hardin 1984, Andrew et al. 1981) were exposed to 2-ethoxyethanol vapours by whole chamber administration by different protocols.

In the study with rabbits 29 inseminated females/group were exposed to 2-ethoxyethanol at concentrations of 0 ppm, 160 $\pm$ 31 ppm ("low level") or 617 $\pm$ 49 ppm ("high level"), 7 h/day on g.d. 1-18. The animals were terminated on g.d. 30 and fetuses of finally 22 to 24 litters were examined for external, visceral and skeletal defects. Food consumption in both 2-ethoxyethanol exposed groups was significantly less than in the control group. In the high level group maternal body weight gain was dramatically reduced and 5 does died during the study. Mean relative liver weights were increased in both exposure groups as was relative kidney weight in the high level group. Based upon the percent of does pregnant at sacrifice, there was no evidence that daily exposure to 2-ethoxyethanol during g.d. 1-18 overtly altered rabbit fertility, however, exposure to 617 ppm resulted in 100% embryomortality as indicated by exclusively early resorption in the uteri of all pregnant does. Also in the 160 ppm group the mean number of resorption per litter and the number of litters with resorption were significantly increased in comparison to the controls. While no effects were detected on fetal size (weight or length) significant increases in the incidence of major malformations (ventral wall defects and fusion of aorta with pulmonary artery), visceral anomalies (renal changes) and skeletal variants (supernumerary ribs with associated vertebral variations and external defects) were observed.

In the study with rats groups of 29 to 38 females were exposed to 2-ethoxyethanol for three weeks (pregestational exposure) at concentrations of 0 ppm, 150 $\pm$ 18 ppm or 649 $\pm$ 50 ppm, 7 hr/day, 5 days/week and/or 0 ppm, 202 $\pm$ 11 ppm or 767 $\pm$ 22 ppm on g.d. 1-19 , 7 hr/day (gestational exposure). The animals were terminated on g.d. 21 and fetuses of finally 28 to 37 litters were examined for external, visceral and skeletal defects. Three weeks of exposure of nonpregnant rats to either pregestational exposure level did not appear to alter food consumption or body weight. Gestational exposure to the higher level lead to some reduced weight gain in the late treatment period (g.d. 17 and 21) only and to a decrease in mean relative dam liver weights. It was reported that exposure to 2-ethoxyethanol did not appear to alter mating behaviour, breeding performance, or fertility as indicated by percentage of pregnant dams at sacrifice. Similar to the study in rabbits, the higher level (767 ppm) of gestational exposure resulted in a significant embryolethal effect (100% resorptions). Resorptions per litter in the gestationally exposed 202 ppm group were also about twice the control value, and fetal body size (weight and length) was significantly reduced at these exposure levels. Gestational exposure to 202 ppm induced an increased incidence of cardiovascular defects (transposed and retrotracheal pulmonary artery) and of skeletal defects (predominantly reduced skeletal ossification and various rib dysmorphologies, e.g. extra and rudimentary ribs, partly associated with thoracic vertebrae). The authors concluded from their study that significant incidences of terata, intrauterine growth retardation, and

embryomortality were induced at levels that were below or were similar to those that induce manifestations of maternal toxicity.

In a study focusing on behavioural teratology (Nelson and Brightwell 1984; Nelson et al. 1981) pregnant Sprague-Dawley rats were exposed to 2-ethoxyethanol vapours at concentrations of 200, 300, 600, 900, and 1200 ppm (7 hr/day) during a range-finding pilot study from either g.d. 7-13 or g.d. 14-20. It was reported that no offspring survived at the 1200 and 900 ppm group and that there were approximately 34% neonatal deaths even after exposure to 200 ppm. Cross fostering to control dams revealed that the cause of neonatal mortality was not due to effects on the mothers. Also duration of pregnancy had been consistently extended in this study for about two days. Behavioural testing and neurochemical evaluations in offspring were performed after prenatal exposure to 100 ppm using the same regimen. Even at this level of exposure there was an increased duration of pregnancy. In the offspring testing numerous deviations from controls were observed for various test conditions (rotorod, open field, activity wheel, avoidance conditioning). Neurochemical evaluation of whole-brain samples from new born pups revealed significantly decreased levels of norepinephrin and regional analyses of brains from 21-day-old offspring revealed significant elevations of various neurotransmitters (acetylcholine, dopamine, 5-hydroxytryptamine) in the cerebrum.

Oral route of administration

With the oral route of exposure there are some less well documented studies available.

In a study with Sprague-Dawley rats (Goad et al. 1984, abstract) sperm-positive females were gavaged with 200 mg 2-ethoxyethanol/kg bw/day during different periods of gestation (g.d. 7-9, 10-12, 13-15, or 5-15). At sacrifice on g.d. 20 the numbers of live and dead implants were counted. Live fetuses were weighed, measured for crown-rump length, and examined for gross, visceral, and skeletal abnormalities. It was reported that 2-ethoxyethanol administration on g.d. 7-15 resulted in a significant decrease in maternal weight gain and an increase in prenatal mortality, with neither of these effects observed with any short-term dosing interval. Short-term administration produced a decrease in fetal weight in all treatment groups, with variable effects on fetal length. Furthermore it produced cardiovascular and skeletal abnormalities. The incidence of cardiovascular anomalies (not further specified) varied from 1 to 24% for the various dosing intervals with no such anomalies observed in the controls.

In a further study (Chester et al. 1986, abstract) 2-ethoxyethanol was given to pregnant rats via drinking water during g.d. 7 to 17 at concentrations of 2.5, 3.0, 3.5, or 4.0 mg/mL. Based upon body weight and fluid intake these concentrations were calculated to have resulted in consumed doses of 210 up to 550 mg/kg bw/day. It was reported that in 15 litters that received between 210 and 270 mg/kg bw/day embryomortality was 31% of implants with no apparent effect on pup body weights. In 19 litters that received 270 to 400 mg/kg bw/day embryomortality was 69% of implants, significantly reduced pup weights (50-89% of the controls) with signs of delayed development, but no malformations were seen. In 8 litters that received 400 to 550 mg/kg bw/day embryomortality was 100% with no signs of maternal toxicity observed.

In an older study on Wistar rats (Stenger et al. 1971) animals were treated with 2-ethoxyethanol by gavage during g.d. 1 to 21 with amounts of 12.5, 25, 50, 100, 200 or 400 µl/kg bw/day (according to 11.5, 23, 46.5, 93, 186 and 372 mg/kg bw/day). No effects were

observed at volumes up to 25 µl/kg bw/day. An increase in the number of early and late prenatal death was observed at doses of 50 µl/kg bw/day and more. Fetal body weights were affected from 100 µl/kg bw/day and more and there was a clear increase in the number of fetuses with skeletal variations and retardation. At 400 µl/kg bw/day the post-implantation loss was about 100%.

2-ethoxyethanol was also investigated in mice using the Chernoff and Kavlock screening bioassay.

In the study of Schuler et al. (1984) pregnant CD-1 mice were orally dosed once per day on g.d. 7 - 14 with a dose of 3 605 mg/kg bw/day. At this dose level maternal mortality was 10% and prenatal mortality was 100%.

The study of Wier et al. (1987) used a modified protocol using different dose levels and including a separate so-called teratology probe. For this latter part 6 pregnant CD-1 mice/group were treated orally once per day on g.d. 8 - 14 with doses of 1 000, 1 800, 2 600, 3 400, and 4 200 mg/kg bw/day. Dams were sacrificed at g.d. 18. Significantly reduced fetal body weight was revealed at the dose of 1 000 mg/kg bw/day. Maternal toxicity (in terms of reduced body weight gain) was observed at 1 800 mg/kg bw/day, also clinical signs and mortality at higher dose levels (3 400 mg/kg bw/day). An increased incidence of resorptions was observed at 1 800 mg/kg bw/day associated with fewer live fetuses at termination. At the higher dose levels (3 400 mg/kg bw/day) embryomortality was about 100%. The mean number of malformed fetuses was significantly elevated for the 1800 and 2600 mg dose groups. The pattern of malformation included cleft palate, exencephaly and fused or missing digits of the forepaw. In the postnatal part of the study for which 20 females/group had been gavaged with 800 or 1 200 mg/kg bw/day external examination of the offspring also revealed malformations of the forepaw and in addition kinked tail. For both dose groups the percentage of pups with kinked tail was observed to increase with postnatal age. In the higher dose group also the mean number of live-born pups was significantly reduced with postnatally increasing mortality.

Dermal route of administration

Developmental toxicity was also investigated by the dermal route of application in Sprague Dawley rats (Hardin et al. 1984). 2-Ethoxyethanol was applied to 18 pregnant dams at a total daily dose of 1.0 mL (0.25 mL 4x/day at 2.5-hr intervals) to the shaved skin at the interscapular region during g.d. 7-16. Taking into account relative density of 0.93 g/ml and with the assumption of a body weight of 200 g and dermal absorption of 20-27% (Sabourin et al., 1992) this dosing may be estimated to yield about 930-1255 mg/kg bw/day. Animals were sacrificed at g.d. 21 and fetuses were evaluated for external, visceral and skeletal examinations. Toxic signs were not noted in the 2-ethoxyethanol treated rats, however, body weight gain was reduced as was gravid uterus weight, the latter accounting for much of the difference in body weight as well as extragestational body weight gain. At sacrifice a significantly higher frequency of completely resorbed litters (7 out of 18) and an increased number of dead implants per litter were observed. The number of live fetuses per litter was reduced, also the body weights of live fetuses were significantly reduced. On gross examination three fetuses with acaudia and imperforate anus were noted. Visceral examinations revealed statistically significant increases in cardiovascular, renal and brain

malformations as well as testicular defects in some of the male offspring. Skeletal examinations revealed statistically significant increases in several skeletal variations (ribs and vertebrae) and skeletal retardation. The administration of 2.0 mL 2-ethoxyethanol (0.50 mL 4x/day at 2.5-hr intervals) which had been already reported earlier (Hardin et al. 1982) resulted in clinical signs of maternal toxicity (ataxia), impaired body and organ weights and in complete resorption of all litters.

In an older study (Stenger et al. 1971) Swiss White mice, Wistar rats, and rabbits (Yellow-silver) had been treated with 2-ethoxyethanol by subcutaneous injection into skin of the back. No embryo-, fetotoxic and teratogenic effects were reported for rabbits and mice treated during g.d. 7-16, resp. 1-18, at volumes of 25 µl/kg bw/day, resp. up to 100 µl/kg bw/day. In rats treated during g.d. 1-21 with doses of 25, 50 and 100 µl/kg bw/day reduced fetal body weight and an increase in skeletal variations and retardation was reported for the 100 µl/kg bw/day dose level.

Mode of action

Numerous *in vivo* and *in vitro* investigations have been demonstrating that the major toxic potential of both 2-ethoxyethanol and of 2-ethoxyethyl acetate is attributable to their joint metabolite 2-ethoxy acetic acid (reviewed by DFG 1993, c.f. BUA 1995), which is finally considered the ultimate toxic agent.

This may also account for the effects adverse to reproduction as indicated by several related experimental *in vivo* and *in vitro* investigations. For review the following citations are taken from BUA 1995:

In vitro studies on cultures of Sertoli- and germ cells showed that ethoxyacetic acid alone is capable of causing degenerations of spermatocytes. Regarding this, ethyl glycol was proven to be ineffective. Parallel to the morphological changes, the activity of a few enzymes of the germ cells was altered (Gray et al. 1985). In an other *in vitro* study, the oxygen consumption and the adenosine-triphosphate concentration in isolated spermatocytes were measured as a function of the application of ethyl glycol and ethoxyacetic acid. A change of the cellular metabolism was determined only under the influence of the alkoxyacetic acid (Oudiz and Zenick 1986). As was shown in the study of liver mitochondria, their metabolism is disturbed by 2-ethoxyacetic acid but not by ethyl glycol (Beattie and Brabec 1986).

Spermatocyte damage was shown in *in vivo* studies on male rats to which ethyl glycol was administered orally. These effects could be fully suppressed, whenever the animals were given substances to inhibit the alcohol metabolism (Foster et al. 1984). This result, too, implicates 2-ethoxyacetic acid as the ultimate toxic agent. The research of Nelson at al. 1984 also indicates the same: With the simultaneous application of ethyl glycol and ethanol to pregnant rats, a reduction of the teratogenic effectivity of ethyl glycol was observed.

Conclusion:

Experimental data from studies with mice demonstrated that 2-ethoxyethanol adversely affects male reproductive organs (testes atrophy) as well as sperm parameters and sperm

morphology. 2-ethoxyethanol was further shown to adversely affect reproductive capability and capacity in both sexes for at least one generation.

A NOAEL (fertility) of approximately 800 mg/kg bw/day was derived from a fertility study in mice after continuous exposure via drinking water (Lamb et al. 1985).

It is however evident from various other studies (c.f. Table 4.1.2.9) using different species and applying different routes of exposure, that 2-ethoxyethanol specifically affects male reproductive organs (testes atrophy) and is spermatotoxic at clearly lower dose/concentration ranges depending on which parameters had been determined.

A NOAEC (male reproductive organ toxicity/ spermatotoxicity) of 100 ppm was derived from a 13 week repeated dose toxicity study in rabbits (Bio/dynamics Inc 1983; Barbee et al. 1984) and a NOAEL (male reproductive organ toxicity/ spermatotoxicity) of 93 mg/kg bw/day was derived from a 13 week repeated dose gavage study in rats (Stenger et al. 1971).A NOAEL (male reproductive effects) of 1250 ppm in drinking water (109 mg/kg bw/d) was established from a 13 week repeated dose study in rats (NTP 1993).

In addition studies on rabbits, rats and mice with the inhalatory, oral and dermal route of exposure consistently demonstrated that 2-ethoxyethanol adversely affects embryonic and fetal development in terms of embryo-/fetomortality, fetal growth retardation and visceral/skeletal malformations and variations in a dose-related manner. Significantly increased incidences of these developmental effects were induced already at dose levels without obvious maternally toxic effects, respectively borderline effects. Comparable effects could be also revealed by use of the dermal route of exposure. The teratogenic effects such as increase in skeletal and cardiovascular malformations were seen predominantly in rats and rabbits, whereas exencephaly and cleft palate were only seen in the mouse.

A NOAEC (developmental toxicity) of 10 ppm was derived from the rat study with inhalation exposure (Doe 1984b; Tinston et al. 1983a). A NOAEL/developmental toxicity of 23 mg/kg bw/day was derived for the oral route from studies with rats (Stenger et al., 1971). A LOAEL/developmental toxicity of 930-1255 mg/kg bw/day was derived for the dermal route from the study of Hardin et al., 1984.

Based on the evaluation of the available animal data classification and labelling as Reprotox. Cat. 2, R 60/R 61 is confirmed.

Human data:

There are data from several epidemiological studies available indicating an association between exposures to 2-ethoxyethanol, respectively 2-ethoxyethyl acetate reproductive disorders.

For the evaluation of a possible associations between exposure to ethylene glycol ethers and impaired fertility a case-control study was conducted among first time patients at a clinic for reproductive disorders (Veulemans 1993). The study group consisted of 1019 cases, defined as patients diagnosed infertile or subfertile on the basis of a spermiogram and 475 controls who were diagnosed as normally fertile by the same procedure. Possible exposure to ethylene glycol ethers was assessed by the presence of the urinary metabolites methoxyacetic acid

(MMA) and ethoxyacetic acid (EAA) respectively for 2-methoxyethanol and 2-ethoxyethanol or their acetates. EAA was detected in 39 patients and in six controls, with a highly significant odds ratio of 3.11 (p= 0.004). The presence of EAA in urine proved to be strongly associated with exposure to preparations containing solvents, especially paint products, and with some groups of occupation, the most important which were also directly or possibly connected with paint products. The association between urinary EAA and diagnosis remained significant even when other industrial spermatotoxic chemicals were as confounders. On dividing the study group according to sperm concentration corrected for motility and morphology, a highly significant clustering of EEA positive patients was found among the subcategories representing complete azoospermia and severe oligozoospermia (cf. Table 4.1.2.9.2). There was no correlation between EAA concentrations and the various measures of sperm quality in this study. This was explained by the authors however by the distorting influence of the latent period between exposure and possible spermatotoxic effects.

Table 4.1.2.9.2

Distribution of EAA positive subjects in subgroups defined by the concentration of sperm with normal motility and morphology (adapted from Veulemans 1993)

Sperm concentration (10^6/mL)	EAA positive subjects	Total subjects
0	11	151
>0 - < 10	24	738
10 - < 20	4	23
20 - < 40	4	205
≤ 40	2	166

These findings are further supported by two other studies on workers occupationally exposed to ethylene glycol ethers.

The effects of exposure to ethylene glycol ethers on male reproduction were evaluated in a cross-sectional study (Welch et al. 1988) consisting of 73 ship yard painters and of 40 controls (non-exposed employees from the same shipbuilding facility). Within an industrial hygiene survey the exposure to ethylglycol and to methylglycol based on an 8-hour time weighted investigation of workplace air concentrations was evidenced for the painters. Skin contact was also anticipated. Workers exposure to glycol ethers was also verified by measuring urinary metabolites, however, data were not presented. The results of the semen analysis of the study participants suggested that there was an effect of exposure to ethylene glycol ethers on sperm count. Although mean values of total sperm count/ejaculate did not significantly differ between exposed and controls ($158x10^6$/mL versus $211x10^6$/mL), biologically meaningful differences were seen when the proportion of men with oligospermia

was examined. The proportion of exposed men with oligospermia (less than or equal to 20 million/cc) was 13% in the exposed group versus 5% expected based on other population surveys, respectively in the unexposed group. The proportion of painters with azoospermia was 5%, with only 1% expected based on other population surveys, respectively 0% in the controls. Among non-smokers the exposed group had a higher rate of oligospermia. The odds ratio for oligospermia among the painters was increased to 2.8 among the non-smokers.

Another cross-sectional study was conducted among men exposed to ethyl glycol used as a binder slurry in a metal casting process in a plant in Portland, Oregon (Ratcliffe et al. 1989). Workers exposure to 2-ethoxyethanol was verified by the investigation of workplace air concentrations and by monitoring urine excretion of the metabolite 2-ethoxyacetic acid (EAA). 37 exposed men and 39 non-exposed controls from elsewhere in the plant provided a sperm sample. The average sperm count per ejaculate among exposed workers was significantly lower than that of the controls ($113x10^6$ versus $154x10^6$ per ejaculate). The mean sperm concentration of the exposed and unexposed group did not significantly differ from each other (44 and $53x10^6$/mL respectively). No effect on semen volume, sperm viability, motility, velocity, and normal morphology or testicular volume was detected, although some differences in the proportion of abnormal sperm shapes were observed. The authors concluded that their findings suggest a possible effect of exposure to 2-ethoxyethanol on sperm counts in these workers, however they would not exclude the possibility that other factors or bias due to low participation rates may have led to these results.

A further study was designed to address the potential association of spontaneous abortion with fabrication room work (fab) in the silicon-based semiconductor industry (Schenker et al. 1995). The study was conducted nation wide at 14 semi-conductor industry companies in the USA. A small increase in the risk of spontaneous abortion was observed among fab workers compared with nonfabrication room (non-fab) workers in two cohorts: historical (adjusted RR = 1.43, 95% confidence interval 0.95-2.09) and prospective (adjusted RR = 1.25, 95% confidence interval 0.65-1.76). Analysis of specific fab exposures in the historical cohort showed a consistent, dose-response association of spontaneous abortions with photoresistent and developer solvents, whose major component was ethylene-based glycol ethers. Association of spontaneous abortions with self-reported stress and with etching fluorides were also observed. No significant decrease in fertility was observed among men or women working in fab rooms.

Furthermore, a retrospective cohort study was conducted among workers at two semiconductor manufacturing plants in the eastern United States in 1980-1989 for determination of whether occupational exposure to ethylene glycol ethers was associated with increased risks in spontaneous abortion and subfertility (Correa et al. 1996). Reproductive and occupational histories were obtained from interviews of semiconductor manufacturing workers and spouses. Assessment of potential exposure to mixtures containing ethylene glycol ethers (none, low, and high) was based on reported processes and company records. 1150 pregnancies (561 to female employees, 589 to wives of male) were evaluated. Among female manufacturers, potential exposure to mixtures containing ethylene glycol ethers was associated with increased risks of spontaneous abortion (high exposure group RR = 2.8, , 95% confidence interval 1.4-5.6) and subfertility (high exposure group OR = 4.6, 95% confidence interval 1.6-13.3). Among spouses of male manufacturers potentially exposed to mixtures containing ethylene glycol ethers, there was no increased risk in spontaneous abortion, but there was a nonsignificant increased risk of subfertility (high exposure group OR = 1.7; , 95% confidence interval 0.7-4.3)

Recently, as part of a multicenter case-control study conducted in six regions in Europe the risk of congenital malformations related to glycol ether exposure during pregnancy was evaluated (Cordier et al. 1997). The study comprised 984 cases of major congenital malformations and 1134 controls matched for place and date of birth. Glycol ether exposure during pregnancy was evaluated using the job description given by the mothers during an interview using a standardized questionnaire. The overall odds ratio (OR) of congenital malformation associated with glycol ether exposure was 1.44 (95% confidence interval 1.10 - 1.90) after adjustment for several potential confounders. From the malformations classified into 22 subgroups the association with exposure to glycol ethers appeared particularly strong for neural tube defects, cleft lip and multiple anomalies.

Conclusion:

Human data from several epidemiological studies also indicate an association between exposure to 2-ethoxyethanol, respectively 2-ethoxyethyl acetate, and impairment of reproduction in male and female humans. From the occupational studies, mainly focusing on spermatotoxic effects, work-related exposures give evidence for a negative influence on sperm count and sperm morphology. The plausibility of the observations from epidemiological studies is supported by the data of the numerous experimental studies, which demonstrated similar effects in laboratory animals.

4.1.3 Risk characterisation

4.1.3.1 General aspects

2-Ethoxyethanol is well absorbed via the respiratory tract, the skin and the gastrointestinal tract. The principle metabolites in the urine are 2-ethoxyacetic acid and ethylene glycol. The glycine conjugate of 2-ethoxyacetic acid also occurs in animals, but not in humans. In animal experiments, 2-ethoxyethanol degradation could be inhibited by ethanol. The main route of excretion is via the urine. Feces and exhaled $^{14}CO_2$ represent minor routes of excretion. The half-life for the excretion of 2-ethoxyacetic acid ranged from 21 h (experimental conditions) to 57 h (work place conditions) for humans, but only 7 to 12.5 h in rats. Respiratory elimination of unchanged 2-ethoxyethanol for humans is $\leq$4% of the total body uptake. The extent of absorption after oral exposure is assumed to be 100 % for risk characterisation purposes (worst case). Based on human and animal data, 50 % dermal absorption should be taken for risk characterisation purposes. Based on human data, 64 % absorption via the inhalation route is recommended for risk characterisation purposes in humans. For animals on the other hand, lower inhalation absorption percentages can be assumed (30 %).

The acute toxicity of 2-ethoxyethanol is low after oral (LD50 (rat) 2300-4700 mg/kg bw), dermal (LD50 (rabbit) 3311-4576 mg/kg bw) and inhalative (LC50 (rat) 15.2 mg/l)

application. These data do not support existing classification and labelling. In consequence, R 20/21/22 should be removed. The substance does not cause local irritation in the skin of rabbits, but causes moderate eye irritation with superficial corneal necrosis in rabbits. 2-Ethoxyethanol does not exhibit skin sensitization in guinea pigs, as judged on the basis of the results of a Magnusson Kligman test.

Repeated dose toxicity studies in experimental animals documented that 2-ethoxyethanol caused hemolytic anemia by inhalation and oral route of exposure. Testicular atrophy, leading to cessation of spermatogenesis, has been observed following exposure to 2-ethoxyethanol by inhalation and oral route of exposure and by subcutaneous injection. 100 ppm (390 mg/m³) is the NOAEC derived from the most sensitive effect observed on the male reproductive system for exposure and uptake of 2-ethoxyethanol via inhalation in New Zealand white rabbits. Effects on the blood and hematopoietic system and male reproductive system also appear to be the most sensitive endpoints for repeated oral exposure. For oral exposure, the NOAEL (rat) was 93 mg/kg bw/day.

The target organs and systems in subacute, subchronic and chronic studies are the proliferating tissues. Repeated exposure of experimental animals to 2-ethoxyethanol by various routes of administration have revealed adverse effects on hematopoietic system. The effects seen are a transient increase in erythrocytic fragility, anemia, and in some instances, leucopenia. Pathological changes have occasionally been observed in the spleen, bone marrow or thymus.

Various types of kidney injury have been reported after high doses. However, in most cases the effects were slight or the studies were very limited and no consistent pattern was evident. Sign of central nervous system dysfunction have been noted after exposure to high levels of 2-ethoxyethanol. There was limited evidence for neurological findings or behavioural effects with lower doses.

2-Ethoxyethanol was negative in bacterial gene mutation tests and in a gene mutation test with mammalian cells. In vitro chromosomal aberration tests (without S-9 mix) and in vitro SCE tests (with and without S-9 mix) were positive at extremely high concentrations (35 mmol/l and higher). Due to these test results the substance seems to have a marginal mutagenic potential for mammalian cells in culture. The negative in vivo micronucleus test indicates that this potential is unlikely to be expressed in vivo.

The findings of the 2-year oral studies in rats support the assumption that there was no indication on carcinogenic effects to 2-ethoxyethanol (NOAEL: drinking water (rat and mice) 1000 mg/kg bw/d and (diet, rat) 900 mg/kg bw/d). No other data were available concerning the carcinogenicity of 2-ethoxyethanol.

Human data from several occupational studies indicate an association between exposure to 2-ethoxyethanol, respectively 2-ethoxyethyl acetate, and impairment of reproductive capability in male and female humans. The plausibility of the observations from epidemiological studies is strongly supported by the data of the numerous experimental studies, which demonstrated similar effects in laboratory animals.

The reproductive toxicity of 2-ethoxyethanol was investigated in a number of studies using oral, inhalation, and dermal routes of exposure involving various animal species. These studies gave clear indications for male reproductive organ toxicity (testes) and for adverse

effects to spermatogenesis. Testicular atrophy, leading to the cessation of spermatogenesis has been seen in rat and rabbits by various application routes. Furthermore, 2-ethoxyethanol was revealed to adversely affect reproductive performance attributable not only to fertility impairment of the male but also of the female sex. Moreover, in developmental studies it was demonstrated that 2-ethoxyethanol exhibits an embryo-/fetotoxic and teratogenic potential. Classification and labelling as Reprotox. Cat. 2, R 60/R 61 is confirmed.

Table 4.1.3.1 Toxicological hazard identification

Substance name	**Inhalation (N(L)OAEL)**	**Dermal (N(L)OAEL)**	**Oral (N(L)OAEL)**
Acute toxicity	LC50/ rat 15.2 mg/l	LD50 / rabbit 3311-4576 mg/kg bw	LD50/ rat 2300–4700 mg/kg bw
Irritation / corrositivity	skin: undiluted, rabbit, no skin irritation eye: Draize test, rabbit, moderate irritation with complete recovery respiratory tract: no data		
Sensitization	skin: no sensitization in guinea pigs (Magnusson Kligman Test) respiratory tract: no data		
Repeated dose toxicity	adverse effects on the hematopoietic system NOAEC 100 ppm = 390 mg/m^3 (rabbit) 13 weeks	no data	NOAEL of 93 mg/kg bw/d (rat) NOAEL of 93 mg/kg bw/d (Beagle dogs) 13 weeks
Mutagenicity	no evidence for mutagenicity from in vitro and in vivo studies		
Carcinogenicity	no data	no data	drinking water rat and mice, 2 years NOAEL of 1000 mg/kg bw rat, diet NOAEL of 900 mg/kg bw
Fertility impairment	testes atrophy and sperm abnormality NOAEC 100 ppm = 390 mg/m^{3,} (rats and rabbits) (13 weeks)	no data	testes degeneration and sperm abnormality NOAEL of 109 mg/kg bw/d (rats) (13 weeks)
Developmental toxicity	NOAEC 10 ppm = 39 mg/m^3 (rats)	LLOAEL 930-1255mg/kg bw/d (rats)	NOAEL 23 mg/kg bw/d (rats)
	Fetal growth retardation and mortality, skeletal malformation without maternal toxicity		

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

For occupational risk assessment of 2-ethoxyethanol the MOS approach as outlined in the revised TGD (Human Health Risk Characterisation, Final Draft) is applied. This occupational risk assessment is based upon the toxicological profile of 2-ethoxyethanol (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

Systemic availability for different routes of exposure

Experimental data from humans and animals for 2-ethoxyethanol show high absorption percentages for the different routes of exposure: According to the chapter 4.1.2.1 on toxico-kinetics, metabolism and distribution the extent of absorption after oral exposure is assumed to be 100% (worst case). Based on human and animal data, 50 % dermal absorption is taken in the risk characterisation. 64 % absorption via the inhalation route is recommended for risk characterisation purposes in humans (experimental human data). However, for animals lower inhalation absorption percentages are assumed (30 %).

Occupational exposure and internal body burden

In table 4.1.3.2.A the exposure levels of table 4.1. are summarised and the route-specific and total internal body burdens are identified. Risk assessment for combined exposure requires the calculation of a total internal body burden; to this end the derived route-specific percentages for absorption are used (64% for inhalation and 50% for dermal exposure).

Table 4.1.3.2.A: Occupational exposure levels and internal body burden (2-ethoxyethanol)

Exposure scenario	Inhalation shift average	Dermal contact shift average		Internal body burden of workers after repeated exposure		
				Inhalation[(1)]	Dermal[(2)]	Combined
	mg/m^3	mg/p/d	mg/kg/d	mg/kg/d		
1. Production and further processing as an intermediate	3	21[(3)]	0.3	0.27	0.15	0.42

[(1)] based on the assumption of 64% inhalative absorption; breathing volume of 10 m^3 per shift
[(2)] based on the assumption of 50% systemic availability of 2-ethoxyethanol after dermal contact
[(3)] EASE (90 % protection by suitable gloves)

MOS Approach

The MOS approach for human risk characterisation is described in detail in the TGD (Human Health Risk Characterisation, Final Draft). The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires to convert the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and humans should be accounted for the calculation of the corrected NOAEL. If route-specific information on oral and inhalation absorption is not available, the TGD recommends to assume a 50% oral absorption and a 100% inhalation absorption. For 2-ethoxyethanol for humans 64% absorption after inhalation is assumed, whereas in animals 30% absorption percentage is taken. After dermal contact 50% absorption is used and 100% absorption after oral exposure is assumed (experimental values).

For occupational risk assessment, the corrected NOAEC for inhalation accounts for the difference of the standard respiratory volume (6.7 m^3) and the respiratory volume for light activity (10 m^3).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are exposure by inhalation and dermal contact.

In addition, for risk assessment of combined exposure (exposure by inhalation and dermal contact) an adequate NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation on the one hand is based on allometric scaling (factor 4 for rats, factor 7 for mice, and factor 2.4 for rabbits). For remaining interspecies differences the TGD proposes an additional factor of 2.5.

For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure. For 2-ethoxyethanol the factor of 2 for an adaptation from subchronic to chronic exposure is used.

The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For 2-ethoxyethanol no further adjustment factors are used in the risk assessment.

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called “critical exposure level”. Concern will be expressed for scenarios with occupational exposure levels higher than the relevant “critical exposure level”.

4.1.3.2.2 Occupational risk assessment

Acute toxicity

Human data regarding the toxicity of 2-ethoxyethanol are sparse. Toxic effects were observed after oral uptake of mixtures of 50 – 200 ml 2-ethoxyethanol. Because no clear dose relationship after inhalation or dermal contact of 2-ethoxyethanol can be drawn from these

case reports by humans, risk assessment for acute toxicity is done on the basis of animal studies.

Inhalation exposure

LC50-values of 15.2 mg/l/4 h and 7.36 mg/l/8 h were determined in rats. Further information on effects in this study in a sub-lethal dose range is not available. Thus considerable uncertainties are connected with the estimation of an acute NAEL based on lethal doses. For risk assessment of acute inhalation toxicity (8-hour exposure) data on 2-ethoxyethanol-induced lethality are considered less relevant than the results from a rat developmental study from Doe (1984). Rats were exposed to about 0, 39, 195 and 975 mg/m^3 2-ethoxyethanol for 6 h/d on gestation day 6-15. There was no evidence for any maternal toxicity at 39 and 195 mg/m^3, whereas at 975 mg/m^3 some slight, but statistically significant haematological changes were observed. A maternal NOAEC of 195 mg/m³ and a LOAEC of 975 mg/m³ was identified.

This experimental value of 195 mg/m^3 has to be converted, because of the different absorption percentage of rat (30%) and human (64%) after inhalation. The external starting point for human lies about 2.13 fold lower than for rats and corresponds to a value of 91 mg/m^3 (195 • 0.3 / 0.64) .

For the identification of the reference MOS, (1) an adjustment factor of 2.5 for interspecies differences (the factor for allometric scaling is already implicitly applied) and (2) a factor of 5 regarding the intraspecies differences for workers are applied.. Multiplying the different adjustment factors, the reference MOS calculates to 12.5 (2.5 • 5). The critical inhalation exposure at the workplace is identified as 7.3 mg/m^3 (91 / 12.5).

There is no concern for scenario 1 (see table 4.1.3.2.B). Keeping in mind that only slight effects were observed at the highest dose of 975 mg/m^3 and the duration of exposure was 10 days, conclusion ii is even more justified.

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

Dermal contact and combined exposure

A dermal LD50 of 3311-4576 mg/kg was determined in rabbits. No further information is available about the dose response relationship in a sublethal dose level.

Based on the before-mentioned line of argumentation, the rat developmental study is used as key study for the assessment (see under inhalation).

The maternal NOAEC of 195 mg/m³ corresponds to an external dermal dose of 56 mg/kg/day (195 mg/m^3 multiplied with the default respiratory volume for the rat for 6 hours of 0.288 mg/kg/day). Taking a dermal absorption of 50 % into account, this external value corresponds to an internal value of 28 mg/kg/day (56 mg/kg/day • 0.5).

For the identification of the reference MOS, (1) a factor of 10 for interspecies differences (a factor for allometric scaling of 4 multiplied with a factor of 2.5 for remaining interspecies differences) and (2) a factor of 5 regarding the intraspecies differences for workers are applied. Multiplying the different adjustment factors, the reference MOS calculates to 50 (4 • 2.5 • 5). The external critical exposure level at the workplace is identified as 1.1 mg/kg/day (56 / 50). The internal critical exposure level gives a value of 0.6 mg/kg/day (28 mg/kg/day / 50).

There is no concern for scenario 1 (see table 4.1.3.2.B). Keeping in mind that only slight effects were observed at the highest dose of 975 mg/m^3 in the test and the duration of exposure was 10 days, conclusion ii is even more justified.

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

Table 4.1.3.2.B: Acute toxicity of 2-ethoxyethanol

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	91 mg/m³			56 mg/kg/day			28ay		
Reference MOS	12.5						-		
Critical exposure level	7.3 mg/m³			1.31 mg/kg/day (external dose)			0.6mg/kg/day (internal dose)		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing in the large-scale industry	3	30	ii	0.3	-187	ii	0.42	-67	ii

Irritation/Corrosivity

Skin/Eye/Inhalation

In a Draize test with rabbits the substance caused mild skin irritation that reversed within 7 days. Draize eye tests with rabbits demonstrated moderate eye irritation that reversed within 10 days. The observed effects are not considered sufficient for classification. There is no concern for dermal or eye irritation at the workplace for 2-ethoxyethanol.

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

Respiratory tract

No respiratory irritation was reported in the acute inhalation studies. In a RDT study by Barbee (1984), no histopathological changes were detected. No such symptoms were reported in the other RDT studies. Thus, with respect to acute local effects on the respiratory tract, airway damage is not anticipated and no concern is expressed.

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

Sensitisation

Skin sensitisation

In a Magnusson Kligman test with guinea pigs no skin sensitisation was observed. No concern is derived.

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

Respiratory sensitisation

No information on the sensitising potential of the substance at the respiratory tract is available. For the time being a valid study to investigate respiratory sensitisation in experimental animals cannot be recommended. However, 2-ethoxyethanol is not suspected to be a potent respiratory sensitizer in humans according to the fact that during all the years of use no notice of specific case reports has been given. There is no concern with respect to respiratory sensitisation at the workplace.

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

Repeated dose toxicity

Local effects

Inhalation exposure and dermal contact

Local effects were not described in the dermal studies and repeated inhalation studies. The only note from a study from Barbee et al. (1984) of "increased incidence of lacrimation and mucoid nasal discharge at all concentrations from week 2 through week 10 " is not robust enough for the risk assessment. In addition, no such findings were reported in any other inhalation toxicity study available for 2-ethoxyethanol.

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

Systemic effects

No information on the effects in humans after repeated exposure to 2-ethoxyethanol is available.

Repeated administration of 2-ethoxyethanol by oral and inhalation routes produced adverse effects in several experimental animals (rats, mice, rabbits and dogs). Target organs are the blood and hematopoietic system and the male reproductive system. The occurred effects seen at the animals are thought to be relevant for man.

Inhalation exposure

There are several inhalation studies with different experimental animals available. The study which is judged to serve as key study for the assessment of inhalation exposure is a 13-week rabbit study. The rabbits were exposed to 0, 25, 100 or 400 ppm 2-ethoxyethanol vapours (equal to 0, 92.5, 390, or 1480 mg/m^3) for 6 h/day, 5 days/week. At 1480 mg/m^3 a weight reduction of testis and slight focal seminiferous tubule degeneration was observed. In addition hematocrit value, hemoglobin concentration and erythrocyte count were decreased. Based on these effects the value of 390 mg/m^3 is taken as NOAEC.

The experimental NOAEC of 390 mg/m^3 is (1) adapted by a factor of 0.46 (0.3 / 0.64) to account for absorption differences after inhalation between experimental animals (30%) and humans (64%). %), (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers and (3) withan for humans . This results in an adjusted inhalation starting point of 90 mg/m^3 (390 • 0.46 • 6.7/10 • 6/8).

The following adjustment factors are applied for the identification of the reference MOS. For (1) interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for (2) intraspecies differences (workers) the default factor is 5, and for (3) duration adjustment a factor of 2 is used. Thus the reference MOS calculates to 25 (2.5 • 5 • 2). The critical inhalation exposure level at the workplace is identified as 4.83.6 mg/m^3 (90 / 25).

The shift average value for inhalation is reported as 3 mg/m^3 for production and further processing of 2-ethoxyethanol. The exposure level in this occupational scenario is lower than the critical inhalation exposure. There is no concern for this scenario. For corresponding MOS values see table 4.1.3.2.C.

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

Dermal contact

Dermal studies with repeated application are not available. Thus studies with other routes of application are taken into account. After viewing of all described studies, the above-mentioned well performed inhalation study is preferred to other described oral gavage or drinking water studies (see 4.1.2.6).

For MOS calculation the NOAEC of 390 mg/m^3 from the above mentioned inhalation study in rabbits has to be transferred into an external dermal dose.

The experimental NOAEC of 390 mg/m^3 is multiplied with wwith the breathing volume of 0.230 m^3/kg/day (0.48 l/min/kg respiratory rate for rabbits • 60 min • 8 h). This gives a value of 45.290 mg/kg/day of inhaled 2-ethoxyethanol (390 mg/m^3 • 0.230 m^3/kg/day). Considering the 30% absorption after inhalation the external value of 90 mg/kg/day corresponds to an uptake of 627 mg/kg/day (internal value). Considering the dermal exposure situation, this internal value has to be multiplied with 2, because the dermal absorption is 50%. This gives an external starting point of 254 (627 • 2). Thus the value of 254 mg/kg/day is taken as starting point for MOS calculation (table 4.1.3.2.C).

The following assessment factors are taken for the calculation of the reference MOS: (1) a factor of 2.4 • 2.5 (rabbit) for interspecies, (2) a factor of 5 for intraspecies differences, and (3) a duration factor of 2 is used. Altogether the reference MOS calculates to 30 (2.4 • 2.5 • 5 • 2) the corresponding critical exposure level calculates to 0.9 mg/kg /day (2 3054 / 60).

The calculated exposure value for dermal contact of 0.3 mg/kg/dayis lower than the critical dermal exposure level of 0.9 mg/kg /day. There is no concern expressed for this scenario. For corresponding MOS values see table 4.1.3.2.C.

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

Combined exposure

The principle of calculation and evaluation of MOS is the same as above for dermal systemic effects. The internal starting point of 627 mg/kg/day is divided by a reference MOS of 30 (see above, dermal exposure) which results in a critical exposure level of 0.45 mg/kg/day. Compared with the exposure value of combined exposure of 0.42mg/kg/day the critical exposure level reaches borderline. However, no concern is derived for scenario 1. The combined exposure values and the respective MOS values are listed in table 4.1.3.2.C.

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

Table 4.1.3.2.C: Repeated dose toxicity of 2-ethoxyethanol (systemic effects)

	Inhalation	Dermal	Combined

Starting point for MOS calculation	90 mg/m³			254 mg/kg/day (external dose)			627mg/kg/day (internal dose)		
Reference MOS	25			60			60		
Critical exposure level	.3.6 mg/m³			0.9 mg/kg/day			0.45 mg/kg/day		
	Exposure (mg/m^3)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing in the large-scale industry	3	30	ii	0.3	180	ii	0.42	64	ii

Mutagenicity

2-Ethoxyethanol was negative in bacterial gene mutation tests and in a gene mutation test with mammalian cells. Positive results from in vitro chromosomal aberration tests and in vitro SCE tests are not taken into consideration because the concentrations were extremely high. The negative in vivo micronucleus test indicates that the substance does not cause clastogenicity in vivo. No concern is derived.

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

Carcinogenicity

Two long-term studies in rats and mice with 2-ethoxyethanol did not give a hind, that the substance is a potent carcinogen. Concern is not derived.

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

Fertility impairment

Human data are available that describe a correlation between the exposure to glycol ethers and subfertility and sperm effects. However workers were exposed to mixtures of substances and quantitative data of a dose response relationship are not described. Thus risk characterisation concerning fertility impairment is based on experimental results.

Inhalation exposure

2-Ethoxyethanol was applied to mice in a multigeneration study via drinking water (800, 1500 und 2600 mg/kg/day). The NOAEL for fertility impairment in this study was 800 mg/kg/day for both sexes. At 1500 mg/kg/day the number of live pubs/litter and proportion of pubs born alive in comparison to controls were decreased. Histopathological investigations did not show any effects in female gonades, while sperms were already affected at 1500 mg/kg/day. However, the lowest dose of 800 mg/kg/day was not checked for sperm effects, therefore, this study is not taken for the risk assessment.

Instead of the above described multigeneration study, the 13 weeks rabbit study, which was also used for the assessment of repeated dose toxicity, is taken for the assessment of fertility impairment. In the rabbit study over 13 weeks (6 hours/day, 5 days/week), a NOAEC of 390 mg/m^3 (100 ppm) was determined. Histopathological effects in gonades were found at the LOAEC of 1480 mg/m^3 (testis weight reduction and slight focal seminiferous tubule degeneration). The NOAEC of 390 mg/m^3 is used for the MOS calculation.

Most of the calculation steps for this endpoint are identical with the calculation of repeated dose toxicity. Therefore at this place the steps are described only shortly to avoid repetition (for detailed calculation steps see under chapter repeated dose toxicity). The experimental NOAEC of 390 mg/m^3 corresponds in an adjusted inhalation starting point of 90 mg/m^3.

Adjustment factors for the identification of the reference MOS are: (1) the default factor of 2.5 for interspecies differences and (2) the default factor of 5 for intraspecies differences (workers). This gives a reference MOS of 12.5 (2.5 • 5). The critical inhalation exposure level at the workplace is identified as 67.2 mg/m^3 (90 / 12.5).

The exposure level for scenario 1 with 3 mg/m^3 is lower than the critical inhalation exposure of 7.2 mg/m³. There is no concern for this scenario. For corresponding MOS values see table 4.1.3.2.D.

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

Dermal contact

Dermal fertility studies are not available. Therefore the above mentioned inhalation study is used for the risk assessment.

For MOS calculation the NOAEC of 390 mg/m^3 is transferred to an external starting point of 54 mg/kg/day (detailed calculation steps are described above under repeated dose toxicity dermal contact).

Assessment factors for the calculation of the reference MOS are: (1) a factor of 2.4 • 2.5 (rabbit) for interspecies differences and (2) a factor of 5 for intraspecies differences. Altogether the reference MOS calculates to 30 (2.4 • 2.5 • 5) the corresponding critical exposure level calculates to 1.8 mg/kg /day (54 / 30).

The calculated exposure value for dermal contact of 0.3 mg/kg/day is lower than the critical dermal exposure level of 1.8 mg/kg /day. There is no concern expressed for this scenario. For corresponding MOS values see table 4.1.3.2.D.

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

Combined exposure

The principle of calculation and evaluation of MOS is the same as above for dermal systemic effects. The internal starting point of 27 mg/kg/day is divided by a reference MOS of 30 (see above, dermal exposure) which results in a critical exposure level of 0.9 mg/kg/day. No concern is derived for this scenario 1.

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

Table 4.1.3.2.D: Fertility impairment of 2-ethoxyethanol

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	90 mg/m³			54 mg/kg/day (external value)			27 mg/kg/day (internal value)		
Reference MOS	12.5			30			30		
Critical exposure level	7.2 mg/m³			1.8 mg/kg/day			0.9 mg/kg/day		
	Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing in the large-scale industry	3	30	ii	0.3	180	ii	0.42	64	ii

Developmental toxicity

Human data are available that describe a correlation between spontaneous abortion and exposure to glycol ethers. However women were exposed to mixtures of substances and quantitative data of a dose response relationship are not described. Thus quantitative risk assessment is based on animal data.

Animal data show embryotoxic and teratogenic effects in several species via different route of application. Developmental effects were induced already at dose levels without obvious maternally toxic effects, respectively borderline effects.

Inhalation exposure

The study which is judged to serve as key study for the risk assessment of developmental effects is the rat inhalation study (whole chamber administration) with 2-ethoxyethanol from (Doe 1984b). In this study 24 female rats/group were exposed to 2-ethoxyethanol at concentrations of 0, 10, 50, or 250 ppm (appr. 38, 190, or 950 mg/m^3), 6 hours/day on g.d. 6-15. There was no evidence for any maternal toxicity at 10 and 50 ppm, whereas at 250 ppm slight haematological changes were observed. Developmental effects were seen at 50 ppm (i.e. unossified cervical centra, partial ossification of the second sternebrae, extra ribs) and 250 ppm (increase in the incidence of late uterine deaths and in the proportion of dams affected). From this study a $NOAEC_{developmental\ effects}$ of 10 ppm (39 mg/m^3) is derived.

The experimental NOAEC of 39 mg/m^3 is (1) adapted by a factor of 0.46 (0.3 / 0.64) to account for absorption differences after inhalation between experimental animals (30%) and humans (64%), (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers and (3) with a factor of 6/8 to account for differences between the experimental inhalation duration of 6 h per day and an average working day for humans of 8 h per day. This results in an adjusted inhalation starting point of 9 mg/m^3 (39 • 0.46 • 6.7/10 • 6/8).

The reference MOS consists of (1) the default factor of 2.5 for interspecies differences and (2) the default factor of 5 for intraspecies differences (workers). This gives a reference MOS of 12.5 (2.5 • 5). The critical inhalation exposure level at the workplace is identified as 0.72 mg/m^3 (9 / 12.5).

There is concern for 2-ethoxyethanol related developmental toxicity in scenario 1. The exposure value 3 mg/m^3 for inhalation is nearly fourfold higher than the corresponding critical exposure level of 0.72 mg/m^3. For corresponding MOS values see table 4.1.3.2.E.

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

Dermal contact

Dermal studies concerning developmental effects are not available. Therefore, the above-mentioned developmental rat inhalation study with the NOAEC of 39 mg/m^3 is taken for MOS calculation.

The experimental NOAEC of 39 mg/m^3 is multiplied with the breathing volume of 0.384 m^3/kg/day (0.8 l/min/kg respiratory rate for rats • 60 min • 8 h). This gives a value of 15 mg/kg/day of inhaled 2-ethoxyethanol (39 mg/m^3 • 0.384 m^3/kg/day). Considering the 30% absorption after inhalation the external value of 15 mg/kg/day corresponds to an uptake of

4.5 mg/kg/day (internal value). Considering the dermal exposure situation, this internal value has to be multiplied with 2, because the dermal absorption is 50%. This gives an external starting point of 9 (4.5 • 2). Thus the value of 9 mg/kg/day is taken as starting point for MOS calculation (table 4.1.3.2.C).

The following assessment factors are taken for the calculation of the reference MOS: (1) a factor of 4 • 2.5 (rat) for interspecies and (2) a factor of 5 for intraspecies differences. Altogether the reference MOS calculates to 50 (4 • 2.5 • 5) the corresponding critical exposure level calculates to 0.18 mg/kg /day (9 / 50).

The critical exposure level of 0.18 mg/kg/day is lower than the dermal exposure value of 0.3 mg/kg /d. There is concern for this scenario. For corresponding MOS values see table 4.1.3.2.E.

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

Combined exposure

The principle of calculation and evaluation of MOS is the same as above for dermal systemic effects. The internal starting point of 4.5 mg/kg/day is divided by a reference MOS of 50 (see above, dermal exposure) which results in a critical exposure level of 0.09 mg/kg/day.

There is concern for combined exposure.

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

Table 4.1.3.2.E: Developmental toxicity of 2-ethoxyethanol

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	9 mg/m³			9 mg/kg/day			4.5 mg/kg/day		
Reference MOS	12.5			50			50		
Critical exposure level	0.72 mg/m³			0.18 mg/kg/day			0.09 mg/kg/day		
	Exposure (mg/m³)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing in the large-scale industry	3	3	iii	0.3	30	iii	0.42	10.7	iii[(1)]

[(1)]conclusion iii already results from inhalation and dermal exposure, therefore no specific concern for combined exposure is indicated

4.1.3.2.3 Summary of occupational risk assessment

As result of occupational risk assessment for 2-ethoxyethanol, concern is risen for developmental toxicity and risk reduction measures have to be initiated. Table 4.1.3.2.F gives an overview about the conclusions of the toxicological endpoints of 2-ethoxyethanol. For the endpoints acute toxicity, irritation, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity and fertility no concern is expressed.

Table 4.1.3.2.F: Endpoint-specific overall conclusions for the occupational risk assessment of 2-ethoxyethanol

Toxicological endpoints		concern
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	ii
	respiratory	ii
Repeated dose toxicity	local, inhalation	ii
	local, dermal	ii
	systemic, inhalation	ii
	systemic, dermal	ii
	systemic, combined	ii
Mutagenicity		ii
Carcinogenicity	inhalation	ii
	dermal	ii
	combined	ii
Fertility impairment	inhalation	ii
	dermal	ii
	combined	ii
Developmental toxicity	inhalation	iii
	dermal	iii
	combined	iii[(1)]

[(1)]conclusion iii already results from dermal exposure and/or inhalation, therefore no specific concern for the combined exposure scenario is indicated

Risk estimation is mainly based on animal inhalation studies. Based on human and animal data, 50 % dermal absorption is taken in the risk characterisation. 64 % absorption via the inhalation route is recommended for risk characterisation purposes in humans (experimental human data). However, for animals lower inhalation absorption percentages are assumed (30 %).

The most important toxicological endpoint is the developmental toxicity of 2-ethoxyethanol.

Tables 4.1.3.2.G (inhalation) and 4.1.3.2.H (dermal contact) try to visualize the risk profile of 2-ethoxyethanol. According to the tables you will find the relatively high risks on the left, the relatively low risks on the right side of the tables.

Table 4.1.3.2.G: Ranking of health risks for workers (inhalation)

Exposure scenario	Exposure level in mg/m³	Developmental toxicity	Repeated dose toxicity, systemic	Acute toxicity	Fertility
		Critical exposure level in mg/m^3			
		0.72	3.6	7.2	7.2
1. Production and further processing in the large scale industry	3	iii	ii	ii	ii

Table 4.1.3.2.H: Ranking of health risks for workers (dermal contact)

Exposure scenario	Exposure level in mg/kg/day	Developmental toxicity	Repeated dose toxicity, systemic	Acute toxicity	Fertility
		Critical exposure level in mg/kg/day			
		0.18	0.9	1.1	1.8
1. Production and further processing in the large scale industry	0.3	iii	ii	ii	ii

4.1.3.3 Consumers

Following the exposure assessment there is no evidence for direct exposure of consumers to 2-ethoxyethanol.

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

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

4.1.3.4 Man exposed indirectly via the environment

The exposure estimations for humans via the environment are summarised in section 4.1.1.4. A total daily intake of 2.67 x 10^{-3} mg/kg bw/d was calculated for the local scenario. The main contribution is the $DOSE_{plant\ shoot}$ with a fraction of 74 % for oral uptake. The regional exposure of 9.38 x 10^{-7} mg/kg bw/d via drinking water is considered to be negligible.

Comparison of exposure and effects

When considering possible risks of 2-ethoxyethanol to human health arising from indirect exposure via the environment the key areas of concerns may be for repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity.

MOS for the local exposure scenario

Repeated dose toxicity

The major target organs and systems for toxicity of 2-ethoxyethanol were the male reproductive system in a number of species and the blood and hematopoietic system in both sexes of experimental animals. Inhalation exposure (up to 90 days) of experimental animals to concentrations of >400 ppm has been shown to lead to adverse effects on blood parameters and testes in rabbits and rats. The NOAEL for effects on the blood and hematopoietic system and male reproductive system after repeated oral administration was 93 mg/kg bw/d in rats (13-week gavage study). Testicular atrophy occurred in mice given oral doses of >1000 mg/kg bw/d 2-ethoxyethanol (5-week gavage study). Based on the data presented, the toxic effects following repeated oral administration of 2-ethoxyethanol were more severe in rabbits and rats than in mice.

Accordingly, the NOAEL of 93 mg/kg bw/d for effects on the blood, hematopoietic and male reproductive systems in rats for the oral route from the 13-week study was used for considerations on the margin of safety (MOS).

Margin of safety

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- Overall confidence in the database

The database taken into account for performing the risk characterization has been evaluated with regard to its reliability, relevance and completeness according to the TGD. For the assessment of the inherent toxicity of 2-ethoxyethanol no specific data gaps were identified.

The data were published in peer reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognized guidelines and to GLP. In addition, there are some early reports without conformance to requirements of the standard testing protocols, and not to GLP, mostly without detailed data or information, or data submitted as abstracts only.

The findings of all studies are not contradictory so that the judgement can be based on the database.

There are no reasons to assume limited confidence.

- Uncertainty arising from the variability in the experimental data

The principal health effects documented in experimental animals exposed to 2-ethoxyethanol were related to reproduction, the hematopoietic system, and in some cases to the nervous system, thymus, and kidneys.

From the studies referenced in sections 4.1.2.6 and 4.1.2.9 it was consistently demonstrated that hematotoxic effects and impaired reproduction and development were the most sensitive and therefore „critical effects“ in experimental animals. In this respect, adverse effects on the male reproductive system occurred at the same exposure levels as adverse hematological effects.

With respect to these effects the overall database allows to conclude on NOAELs/NOAECs for 2-ethoxyethanol not only from one single study, but from several studies in which similar responses were demonstrated across different species (rabbit, rat, mouse) and independent from the route of application (inhalation, oral, dermal).

There are no reasons to assume a special extent of uncertainty which has to be taken into account.

- The nature and severity of the effect

The hematopoietic system of both sexes, endpoints comprising reproduction, and the developing organism were identified as the most sensitive targets for toxicity of 2-ethoxyethanol.

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Findings described as hematotoxic, as testes effects and as impaired development are considered to be important because they are indications of severe organ damage/dysfunction, respectively severe morphogenetic dysfunction.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Therefore there is concern, which has to be expressed in the magnitude of the MOS.

- Intra- and interspecies variation

Toxicodynamics: The intra- and interspecies variability of toxicodynamic responses have been described in sections 4.1.2.6 and 4.1.2.9.

With respect to the „critical effects“ related to spermatogenesis, male reproductive organs and development, all species tested so far responded in the same way. As far as comparable studies are available, rats appeared to be more sensitive than mice for the oral route of exposure.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Therefore there is concern, which has to be expressed in the magnitude of the MOS.

Toxicokinetics: Data on the kinetics of 2-ethoxyethanol do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. However, the available data give a hint on a particular high variability in kinetics. 2-Ethoxyethanol is well absorbed via the respiratory tract, the skin and the gastrointestinal tract. The principle metabolites in the urine are 2-ethoxyacetic acid and ethylene glycol. The glycine conjugate of 2-ethoxyacetic acid also occurs in animals, but not in humans. In animal experiments, 2-ethoxyethanol degradation could be inhibited by ethanol. The main route of excretion is via the urine. Feces and exhaled $^{14}CO_2$ represent minor routes of excretion. The half-life for the excretion of 2-ethoxyacetic acid ranged from 21 h (experimentally conditions) to 57 h (work place conditions) for humans, but only 7 to 12.5 h in rats.

Since 2-ethoxyacetic acid is considered to represent the ultimate toxicant, the species-specific differences in elimination half-life of this metabolite may be of relevance. This should be taken into consideration for the risk assessment of humans.

- Dose response relationship

No special concern has to be raised with respect to dose response relationship.

- Differences in exposure (route, duration, frequency and pattern)

With respect to the „critical effects“ no qualitative changes in the toxicodynamic response pattern was observed in dependence of the route of exposure. The estimated total daily intake (with an assumed absorption of 100%) is compared with an oral NOAEL from a 13-week study. There are no reasons to assume that special concern can be derived from this procedure..

- the human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for the local exposure scenario

The daily intake was calculated to be 0.00267 mg/kg bw/d. The margin of safety between the

exposure level of	0.00267 mg/kg bw/d

and the

oral NOAEL of	93 mg/kg bw/d

is judged to be sufficient. Thus, with respect to repeated dose effects 2-ethoxyethanol is considered to be of no concern in relation to local indirect exposure 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

Mutagenicity

2-Ethoxyethanol was negative in bacterial gene mutation tests and in a gene mutation test with mammalian cells. In vitro chromosomal aberration tests (without S-9 mix) and in vitro SCE tests (with and without S-9 mix) were only positive at extremely high concentrations. Thus, the substance seems to have a marginal mutagenic potential for mammalian cells in culture. However, the negative in vivo micronucleus test indicates that this potential is unlikely to be expressed in vivo. Concern is not derived.

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

Carcinogenicity

The findings of the 2-year oral studies in rats and mice gave no indication on carcinogenic effects of 2-ethoxyethanol (NOAEL on rats and mice (drinking water) of 1000 mg/kg bw/d and on rats (diet) 900 mg/kg bw/d).

The margin of safety between the NOAEL of 900 mg/kg bw/d based on data from the diet study on rats and the daily intake of 0.00267 mg/kg bw/d is judged to be sufficient to conclude on no concern for tumor formation in relation to local indirect exposure via the environment (MOS of about 3×10^5).

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

Toxicity for reproduction

Effects on fertility

Testicular atrophy, decline in sperm count, abnormal sperm motility and morphology, and degeneration and atrophy of seminiferous tubules in male adult rabbits, rats, mice, and dogs have been observed following exposure to 2-ethoxyethanol through both the inhalation and the oral route. It was also demonstrated that part of these effects were irreversible and persisted for longer periods even after cessation of exposure.

While overall reproductive performance and capability was not adversely influenced at dose levels of up to approximately 800 mg/kg bw/day (drinking water study with mice; Lamb et al. 1985) testicular organ and sperm toxicity was revealed at clearly lower exposure levels with a NOAEC of 100 ppm (inhalation exposure to rabbits, Bio/dynamics Inc 1983; Barbee et al. 1984) or a NOAEL of 93 mg/kg bw/d (gavage study in Wistar rats, Stenger et al. 1971)**.** The NOAEL for testes toxicity (93 mg/kg bw/d) is derived from the 13-week study on Wistar rats.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty which has to be taken into account. The findings of all studies on 2-ethoxyethanol are not contradictory (cf. 4.1.2.6, 4.1.2.9 and 4.1.3.4 Repeated dose toxicity).

- Overall confidence in the database

There are no reasons to assume no confidence.

- Intra- and interspecies variation

Toxicokinetics: Data on kinetics of the substance do not allow to calculate the intra- and interspecies variability by applying modern approaches. However, the available data give a hint on a particular high variability in kinetics (cf. 4.1.3.4 Repeated dose toxicity). The half-life for the excretion of 2-ethoxyacetic acid as main toxic metabolite is longer in humans (range from 21 to 57 h) than in rats (7 to 12.5 h). This species-specific differences may be of relevance for risk characterisation purposes and should be taken into consideration for the risk assessment of humans.

Toxicodynamics: The intra- and interspecies variability of toxicodynamic responses have been described in sections 4.1.2.6 and 4.1.2.9. With respect to the „critical effects“ related to

spermatogenesis, male reproductive organs and development, all species tested so far responded in the same way. As far as comparable studies are available, rats appeared to be more sensitive than mice for the oral route of exposure. There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Therefore there is concern, which has to be expressed in the magnitude of the MOS.

- Dose response relationship

Related to toxicity to reproduction the above indicated NOAELs for fertility and testes toxicity/spermatotoxicity were all derived from well performed multiple dose studies and based on the observation of dose-related changes in specific parameters. No special concern has to be raised with respect to dose response relationship.

- Nature and severity of the effects

Testicular atrophy, decline in sperm count, abnormal sperm motility and morphology, and degeneration and atrophy of seminiferous tubules in male adult rabbits, rats, mice, and dogs have been observed following exposure to 2-ethoxyethanol through exposure via inhalation and oral route. A part of these effects were irreversible and persisted for longer periods even after cessation of exposure. Accordingly, these effects have to be considered as severe health effects (classification as Reprotox. Cat. 2, R 60).

- Differences in exposure (route, duration, frequency and pattern)

The estimated total daily intake with an assumed absorption rate of 100% is compared with an oral NOAEL of 93 mg/kg bw/d for fertility. There are no reasons to assume a special concern from this procedure.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for children.

- Other factors

There are no further factors known requiring a peculiar margin of safety.

MOS for the local exposure scenario

The calculated daily intake is 0.00267 mg/kg bw/d. The margin of safety between the

exposure level of	0.00267 mg/kg bw/d

and the

NOAEL (oral) of	93 mg/kg bw/d

is judged to be sufficient. Thus, with respect to fertility effects the substance is considered to be of no concern in relation to local indirect exposure 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

Developmental toxicity

Besides the response in adults also the developing organism revealed to be very sensitive in response to 2-ethoxyethanol. Embryo/fetal mortality, fetal growth retardation, and visceral/ skeletal malformations have been observed after exposure of pregnant rabbits, rats, and mice to 2-ethoxyethanol by inhalation, oral or dermal application. From an inhalation study on rats a NOAEC for developmental toxicity of 10 ppm was derived (Doe 1984b; Tinston et al. 1983a). The value of -23 mg/kg bw/d from the gavage study on Wistar rats (Stenger et al., 1971) will be used as oral NOAEL for risk characterisation of developmental toxicity.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data

There are no reasons to assume an important uncertainty which has to be taken into account. The findings of all studies are not contradictory (cf. 4.1.2.9 and previous comments in this section).

- Overall confidence in the database

There are no reasons to assume no confidence (cf. previous comments in this section).

- Intra- and interspecies variation

Data on toxicokinetics of the substance do not allow to calculate the intra- and interspecies variability by applying modern approaches. The intra- and interspecies variability of toxicodynamic responses have been described in sections 4.1.2.6 and 4.1.2.9 (cf. also previous comments in this section)

- Dose response relationship

The above indicated NOAECs/NOAELs for developmental toxicity were all derived from well performed multiple dose studies and based on the observation of dose-related changes in specific parameters. There is no reason to assume a special concern.

- Nature and severity of the effects

Embryo/fetal mortality, fetal growth retardation, and visceral/skeletal malformations which have been observed after exposure of pregnant rabbits, rats, and mice to 2-ethoxyethanol by different application routes are considered as severe health effects (classification as Reprotox. Cat. 2, R 61)

- Differences in exposure (route, duration, frequency and pattern)

The estimated total daily intake with an assumed absorption rate of 100% is compared with an oral NOAEL of 23 mg/kg bw/d for developmental toxicity. There are no reasons to assume a special concern from this procedure.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for the local exposure scenario

The calculated intake is 0.00267 mg/kg bw/d. The margin of safety between the

exposure level of	0.00267 mg/kg bw/d

and the

NOAEL (oral) of	23 mg/kg bw/d

is judged to be sufficient. Thus, with respect to developmental effects the substance is considered to be of no concern in relation to local indirect exposure 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

MOS for the regional exposure scenario

Given the negligible exposure for the regional scenario there will be no concern in relation to repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity due to regional indirect exposure of humans to 2-ethoxyethanol 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

4.1.3.5 (Combined exposure)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

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

4.2.2.1 Explosivity

4.2.2.2 Flammability

4.2.2.3 Oxidising potential

4.2.3 Risk characterisation

4.2.3.1 Workers

4.2.3.2 Consumers

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

( ) i) There is need for further information and/or testing

(x) 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

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

Summary of conclusions:

Environment

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

Based on the available data, 2-ethoxyethanol represents no risk to the environment resulting from production, processing, formulation and use.

Workers

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

Concern is derived for developmental toxicity. The corresponding critical exposure level of 0.72 mg/m^3 for inhalation resp. 0.18 mg/kg/day for dermal contact are threefoldlower, than the exposure values of scenario 1 (production and further processing in the large scale industry) for inhalation (3 mg/m^3) and dermal contact (0.3 mg/kg/day).

Consumers

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

Humans exposed via the environment

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

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