Annex VI Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: 2-Ethoxyethanol

EC Number: 203-804-1

CAS Number: 110-80-5

Submitted by: **Germany**

Version/Date: **August 2010**

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: 2-Ethoxyethanol

EC Number: 203-804-1

CAS number: 110-80-5

Registration number (s):

Purity: > 99 % w/w

Impurities and Additives are confidential information, please refer to the confidential Annex.

Proposed classification based on Directive 67/548/EEC criteria:

It is proposed to delete R21 (Harmful in contact with skin) of the current classification.

Current classification remains otherwise unaffected.

Proposed classification based on GHS criteria:

It is proposed to delete Acute Tox. 4*, H312 (Harmful in contact with skin) from the current classification.

Current classification remains otherwise unaffected.

JUSTIFICATION

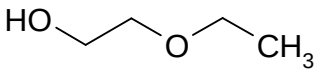
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

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 1.3.

1.1 Name and other identifiers of the substance

Chemical Name:	2-Ethoxyethanol
EC Name:	2-Ethoxyethanol
CAS Number:	110-80-5
IUPAC Name:	2-Ethoxyethanol

1.2 Composition of the substance

Chemical Name:	2-Ethoxyethanol
EC Number:	203-804-1
CAS Number:	110-80-5
IUPAC Name:	2-Ethoxyethanol
Molecular Formula:	C4H10O2
Structural Formula:	
Molecular Weight:	90.1 g/mol
Typical concentration (% w/w):	> 99 % w/w
Concentration range (% w/w):	> 99 % w/w

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	Colourless liquid at 20°C	
VII, 7.2	Melting/freezing point	3.2	< - 80 °C	Ullmann, 1978
VII, 7.3	Boiling point	3.3	132 - 137 °C at 1013hPa	Ullmann, 1978
VII, 7.4	Relative density	3.4 density	0.930 at 20 °C	Ullmann, 1978
VII, 7.5	Vapour pressure	3.6	5.3 hPa at 20 °C	Kirk-Othmer, 1980
VII, 7.6	Surface tension	3.10	69.5 mN/m at 25 °C [1)]	Union Carbide, 1998
VII, 7.7	Water solubility	3.8	miscible in each ratio at 20 °C	Kirk-Othmer, 1980
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log Pow –0.54 to –0.10 [2)]	Dearden & Bresnen, 1988
VII, 7.9	Flash point	3.11	40 °C (closed cup)	Chemsafe, 1996
VII, 7.10	Flammability	3.13	flammable [3)]	Chemsafe, 1996
VII, 7.11	Explosive properties	3.14	not explosive [4)]	Chemsafe, 1996
VII, 7.12	Self-ignition temperature		235 °C	Chemsafe, 1996
VII, 7.13	Oxidising properties	3.15	no oxidising properties [5)]	Chemsafe, 1996
VII, 7.14	Granulometry	3.5		
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17		
XI, 7.16	Dissociation constant	3.21		
XI, 7.17,	Viscosity	3.22		
	Auto flammability	3.12		
	Reactivity towards container material	3.18		
	Thermal stability	3.19		
	Henry Law constant:	5.4.2	0.048 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

2 MANUFACTURE AND USES

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Table 2: Entry of 2-ethoxyethanol in Table 3.1 of Annex VI of EC regulation (no.) 1272/2008 as amended by the 1st ATP

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
603-012-00-X	2-ethoxyethanol; ethylene glycol monoethyl ether	203-804-1	110-80-5	Flam. Liq. 3 Repr. 1B Acute Tox. 4 * Acute Tox. 4 * Acute Tox. 4 *	H226 H360FD H332 H312 H302	GHS02 GHS08 GHS07 Dgr	H226 H360FD H332 H312 H302			

Table 3: Entry of 2-ethoxyethanol in Table 3.2 of Annex VI of EC regulation (no.) 1272/2008 as amended by the 1st ATP

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
603-012-00-X	2-ethoxyethanol; ethylene glycol monoethyl ether	203-804-1	110-80-5	R10 Repr. Cat. 2; R60-61 Xn; R20/21/22	T R: 60-61-10-20/21/22 S: 53-45		E

3.2 Self classification(s)

This should include the classification, the labelling and the specific concentrations limits. The reason and justification for no classification should be reported here.

It should be stated whether the classification is made according to Directive 67/548/EEC criteria or according to GHS criteria.

4 ENVIRONMENTAL FATE PROPERTIES

Not evaluated for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

2-Ethoxyethanol is well absorbed via the respiratory tract, the skin and the gastrointestinal tract (EU RAR, 2008). 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. Elimination via faeces (minor route) and exhalation via lungs (as unchanged compound or – to greater extent – as CO_2) represent further routes of excretion. The half-life for the excretion of 2-ethoxyacetic acid ranged in humans from 21 h (experimentally conditions) to 57 h (work place conditions), 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 and dermal exposure is assumed to be 100 % for risk characterisation purposes. Based on human data an absorption extent of 64 % is recommended for inhalation exposure.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

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. 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).

Animal data

2-Ethoxyethanol has demonstrated acute oral toxicity in several studies with rats, mice and guinea pigs revealing oral LD50 values of 1275-4700 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 as single dose application, 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 LD50 between 4.0 and 5.0 ml/kg, for "diluted" solutions 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"[1] (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

[1] " Cellosolve" is the original trade name of 2-EE (Waite and Patty, 1930). In recent listings of the US-EPA (IRIS) and US-OSHA, the synonyms "cellosolve", "cellosolve solvent" and "ethyl cellosolve" are given. Accordingly the used test substance should be identical and the observed association of substance name and results must be causal. Maybe, purity is different. However, there are no data to support this presumption.

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, 1983a).

An oral LD50 of 1275 mg/kg bw/d in rabbits and of 2350 mg/kg in rats was reported for "ethylcellosolve" ($C_4H_{10}O_2$) in a Russian study (Jazyna et al., 1988). No details on the study were provided.

Conclusion: Based on an oral LD50 value of 1400 mg/kg obtained for guinea pigs and a LD50 of 1275 mg/kg reported for rabbits existing classification as 'Harmful if swallowed' and labelling with R22 is warranted.

5.2.2 Acute toxicity: inhalation

A study with rats revealed for "Cellosolve"[1] after a single 8-hours inhalation exposure a LC50 value of 7.36 mg/l (4.01-13.5 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 female rats. (Pozzani et al. 1959).

Acute inhalation toxicity of 98% pure 2-ethoxyethanol was assessed within the framework of a study on the inter-laboratory reproducibility of OECD TG 403. A maximum non-lethal exposure period (14 days observation post application) for rats was determined in 6 different laboratories after inhalation exposure to ethyl glycol at saturated vapour concentration. 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). This concentration was survived by 10/10 rats when exposed for 3 hours. Details on clinical signs were not provided (Klimisch et al. 1988).

In an inhalation hazard test with 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 cm³ 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). This study had contributed as one of the participants to the review of Klimisch et al. 1988.

In a test using CELLOSOLVE Solvent[1] (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, 1983a).

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 inhalation 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. Evenly distributed 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 dyspnoea 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. 1943a).

Conclusion: The existing classification as "Xn - Harmful by inhalation" and labelling with R20 is confirmed.

5.2.3 Acute toxicity: dermal

In a test using CELLOSOLVE Solvent[1] (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 reported 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, 1983a).

Conclusion: For acute dermal toxicity no classification is required. The current classification with R21 should be deleted.

5.2.4 Acute toxicity: other routes

5.2.5 Summary and discussion of acute toxicity

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. Oral LD50 values in guinea pigs and in rabbits were reported to be 1400 mg/kg and 1275 mg/kg, respectively. The lowest inhalation LC50

value was reported for female rats (7.36 mg/l/8 hours), and dermal LD50 values of 3720 -4576 mg/kg bw were reported for male respectively female rabbits.

Criteria for **acute toxicity by oral route** - Category 4: 300 mg/kg body weight < ATE ≤ 2000 mg/kg body weight		
Reference	Species	LD 50
Smyth et al. 1941	Guinea pig	1400 mg/kg body weight
Jazyna et al. 1988	Rabbit	1275 mg/kg body weight
Criteria for **acute toxicity by inhalation route** - Category 4: 10,0 mg/l < ATE ≤ 20,0 mg/l (based on 4 hour testing exposures)		
Reference	Species	LC 50
Pozzani et al. 1959	Female Rat	7.36 mg/l/8 hours
Criteria for **acute toxicity by dermal route** - Category 4: 1000 mg/kg body weight < ATE ≤ 2000 mg/kg body weight		
Reference	Species	LD 50
Union Carbide 1983a	Male Rabbit	3720 mg/kg body weight
Union Carbide 1983a	Female Rabbit	4576 mg/kg body weight

5.3 Irritation

5.3.1 Skin

5.3.2 Eye

5.3.3 Respiratory tract

5.3.4 Summary and discussion of irritation

C&L including weight-of-evidence considerations.

5.4 Corrosivity

5.5 Sensitisation

5.5.1 Skin

5.5.2 Respiratory system

5.5.3 Summary and discussion of sensitisation

C&L including weight-of-evidence considerations.

5.6 Repeated dose toxicity

Human data:

In exposed workers (painters) in ship industry anemia and leucopoenia have been described. However, these persons were exposed to mixtures with other solvents and heavy metals (Welch, 1988).

Animal data

(*Studies with data on reproductive organs were reported only, full data set on repeated dose toxicity, see RAR on 2-ethoxyethanol, when published* http://ecb.jrc.ec.europa.eu/esis/).

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-Ethoxyacetic acid is formed by enzymatic (alcohol dehydrogenase) oxidation of the free primary hydroxyl group of 2-ethoxyethanol and is finally excreted in urine (Illing and Tinkler, 1985). The toxicity of 2-ethoxyethanol in animals is based on the metabolite 2-ethoxyacetic acid.

5.6.1 Repeated dose toxicity: oral

Table 4: Repeated dose toxicity: oral

Dose Groups, Purity, Exposure route, Species Sex Study Limits	**Exposure duration**	**Adverse effects**	**NOAEL**	**Reference**
0, 250, 500, 1000 mg/kg bw/day*, killed on day 2, 4, 7, or 11 by gavage Sprague-Dawley **rat** (36 m/group) (no data on hematology and clinical chemistry, limited histopathology),	daily for up to 11 days	500 mg/kg bw/day: ↓ sperm count, testis weight changes in sperm motility, testicular degeneration seen in the later stages of primary spermatocyte development and secondary spermatocytes	250 mg/kg bw/day	Foster et al. 1983, 1984
0, 1800 mg/kg bw/d; commercial product by gavage Wistar **rats** (5 m/group) (no data on clinical chemistry, histopathology only on testes and thymus)	10 days	1800 mg/kg bw/day: ↓ massive depletion of leucocyte and thrombocyte numbers RBC, haemoglobin, MCHC; MCV hematocrit, ↓ testes size and weights ↓ thymus: strong involution	-	Ma-Hock et al. 2005

0, 500, 1000, 2000, 4000 mg/kg bw/d* by gavage JCL-ICR **mouse** (5/sex/group) (limited histopathology)	5 days/week 5 weeks	≥ 1000 mg/kg bw/day: ↓ testes weight 2000 mg/kg bw/day: ↓ white blood cell counts, testicular atrophy, tubular degenerative, hypospermia 4000 mg/kg bw/day: mortalities 10/10	500 mg/kg bw/day	Nagano et al. 1979
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 by gavage Wistar **rat** (5/sex/group) (limited data on hematology (no data on RBCs), clinical chemistry, histopathology)	daily for 13 weeks	186 mg/kg bw/day: ↓ hemoglobin concentrations (-9%) ↓ packed cell volume (- 4%) ↑ splenic hemosiderin testes: interstitial oedema and maturation arrest of spermatogenesis	93 mg/kg bw/day	Stenger et al. 1971
0, 50, 100, 200 µl/kg bw/day* (0, 46, 93, 186 mg/kg) by gavage Beagle **dog** (6 animals total, both sexes) (limited data on hematology (no data on RBCs),	daily for 13 weeks	186 mg/kg bw/day: ↓ hemoglobin level (-15%) ↓ hematocrit values (-24%) kidney: distension and flattening of the distal and convoluted tubules in 50% of the dogs testes: degenerative changes in 3/3	93 mg/kg bw/day	Stenger et al. 1971

clinical chemistry, histopathology)				
0, 500, 1000, 2000 mg/kg bw/day (purity >99%) by gavage F344/N **rat** (50/sex/group) (no data on hematology, clinical chemistry, urinalysis, or histopathology)	5 days/week 103 weeks	≥ 500 mg/kg bw/day: adrenal gland enlargement (m) 2000 mg/kg bw/day: terminated at week 17/18 due to high rates of mortalities due to stomach ulcers testes atrophy	-	Melnick 1984
0, 500, 1000, 2000 mg/kg bw/day (purity >99%) by gavage B6C3F1 **mouse** (50/sex/group) (no data on hematology, clinical chemistry, urinalysis, or histopathology)	5 days/week 103 weeks	≥ 500 mg/kg bw/day: testis atrophy 2000 mg/kg bw/day terminated at week 17/18 due to high rates of mortalities due to stomach ulcers (m)	-	Melnick 1984
1.45% (900 mg/kg bw/day)* in feed **rat** (no data on hematology and clinical chemistry)	daily for 2 years	1.45% (900 mg/kg bw/day): renal tubular atrophy, focal fibrosis testes: enlargement of testes, tubular atrophy, interstitial oedema	-	Morris et al. 1942
0, 1250, 2500, 5000, 10000, 20000 ppm	daily for 13 weeks, additional	≥1250 ppm: ↓ water consumption, body weight gain	NOAEL 1250 ppm (109 mg/kg	NTP 1993

(0, 109, 205, 400, 792, 2240 mg/kg in males, and 0, 122, 247, 466, 804, 2061 mg/kg bw/d for females (purity 99%) in drinking water F344/N **rat** (10/sex/group) (method similar to OECD TG 408)	groups of 30 or 56 days of recovery	≥2500 ppm: thymus atrophy (m) abnormal sperm morphology, hypospermia prostate atrophy ≥5000 ppm: ↓ body weight gain, ↓ final mean body weights ↓ absolute and relative weights of testis (non-reversible) mild anaemia (macrocytic, hypchromic) (RBCs -8% (m), -4% (f)), leucopenia at wk 1 + 3, (f) ↑ hematopoiesis in spleen (m) testes degeneration in 6/10 m at 30-d recovery and in 7/10 m at 56 d recovery ↓ epididymis weight ≥10000 ppm: marked hemolytic anemia (RBC -46% (m), - 31% (f)), leucopoenia at wk 1 + 3, marked leucocytosis at wk 13 (m, f), ↑ hematopoiesis in spleen (m) and liver (m/f), Kupffer cell pigmentation, bone marrow hyperplasia, ↓ testis size, ↓ absolute and relative weights of epididymis (non-reversible), thymus atrophy (f), moderate-marked testis degeneration (non-reversible), epididymis aspermia, prostate atrophy, uterus atrophy 20000 ppm: mortalities of 5 males and 7 females in week 8 and 9, treatment ceased, spleen pigmentation & lymph follicle atrophy, liver degeneration, atrophy of bone marrow, thymus, peripheral lymph nodes,	bw/d) for males, NOAEL 2500 ppm (205 mg/kg bw/day) for females	

		atrophy of clitoral gland, ovary, uterus, vaginal epithelium		
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) (purity 99%) in drinking water B6C3F1 **mouse** (10/sex/group) (purity 99%) (method similar to OECD TG 408)	daily for 13 weeks	≥ 10000 ppm: adrenal gland: zona reticularis hypertrophy (f) ≥ 20000 ppm: ↓ body weight gain, emaciation, ↑ hematopoiesis in spleen (f), ↓ absolute testis weights, abnormal sperm morphology, hypospermia 40000 ppm: ↑ hematopoiesis in spleen (m), atrophy of testes & epididymides, testis degeneration	5000 ppm (1304 mg/kg bw/day for females) 20000 ppm (5123 mg/kg bw/day)	NTP 1993

* no data on purity, ↑ increase; ↓ decrease

5.6.2 Repeated dose toxicity: inhalation

Table 5: Repeated dose toxicity: inhalation

Exposure concentrations Species Sex Study Limits	**Exposure duration**	**Adverse effects**	**NOAEC**	**Reference**
0, 25, 100, 400 ppm (0, 92.5, 390, 1480 mg/m³) (whole body), New Zealand White **rabbit** (10/sex/group))	6 hours/day 5 days/week 13 weeks	400 ppm: ↓ hematocrit, ↓ hemoglobin concentration, ↓ erythrocyte counts, ↓ testicular weights, slight focal seminiferous tubule degeneration in 3/10	100 ppm (390 mg/m^3)	Barbee et al. 1984, Bio/dynamics Inc 1983

↑ increase; ↓ decrease

5.6.3 Repeated dose toxicity: dermal

No data available.

5.6.4 Other relevant information

Table 6: Other relevant information

Dose Groups, Exposure route, Species	Exposure duration	Adverse effects	NOAEL	Reference
0, 100, 200, 400, 800 µl/kg* bw/day) (0, 93, 186, 372, 744 mg/kg bw/day) Subcutaneous application Wistar **rat** (5/sex/group)	daily for 4 weeks	186 mg/kg bw: ↓ body weight gain (f) 372 mg/kg bw/day: dyspnoea, somnolence, slight ataxia liver: lobular dissociation, intracytoplasmatic vacuoles kidney: oedema of the tubular epithelium; testes: maturation arrest of testes: spermatogenesis, interstitial oedema and polynuclear cell infiltration 744 mg/kg bw/day: Low food intake	186 mg/kg bw/day (m) 93 mg/kg bw/day (f)	Stenger et al. 1971

* no data on purity, ↑ increase; ↓ decrease

5.6.5 Summary and discussion of repeated dose toxicity

No appropriate human data were available.

In experimental animals, the most prominent adverse effects related to repeated exposures to 2-ethoxyethanol were evident in the hematopoietic system 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 hematopoietic system

Mild hemolytic anemia and corresponding indirect effects such as increased hemosiderin deposition in the spleen and intensified extramedullary hematopoiesis were observed in a number of studies

that included at least a basic set of hematology 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 anemia 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, 1943b) 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 hematopoietic 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 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 5.9.1.

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) (NOAEL 93 mg/kg bw/day). 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 (NOAECL 100 ppm) (Barbee et al., 1984, Bio/Dynamics Inc., 1983).

As a unique finding, uterus atrophy was reported at toxic doses of 20000 ppm in drinking water (2061 mg/kg bw/day) (NTP, 1993):

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.

5.7 Mutagenicity

5.7.1 In vitro data

5.7.2 In vivo data

5.7.3 Human data

5.7.4 Other relevant information

5.7.5 Summary and discussion of mutagenicity

C&L, dose-response estimation including weight-of-evidence considerations.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

5.8.2 Carcinogenicity: inhalation

5.8.3 Carcinogenicity: dermal

5.8.4 Carcinogenicity: human data

5.8.5 Other relevant information

5.8.6 Summary and discussion of carcinogenicity

C&L, dose-response estimation including weight-of-evidence considerations.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Human data:

There are data from several epidemiological studies indicating an association between exposure to 2-ethoxyethanol and reproductive effects.

For the evaluation of 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 et al., 1993). The study group consisted of 1019 cases, defined as patients diagnosed infertile or subfertile on the basis of a spermiogram and 475 controls that 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 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 considered as confounders. On dividing the study group according to sperm concentration corrected for motility and morphology, a highly significant clustering of EAA positive patients was found among the subcategories representing complete azoospermia and severe oligozoospermia (cf. Table 5.9.1.A). 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.

Distribution of EAA positive subjects in subgroups defined by the concentration of sperm with normal motility and morphology

Table 7: Distribution of EAA positive subjects in subgroups defined by the concentration of sperm with normal motility and morphology (adapted from Veulemans et al., 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 and Cullen, 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 2-ethoxyethanol 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 oligozoospermia (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 oligozoospermia. The odds ratio for oligozoospermia among the painters was increased to 2.8 among the non-smokers.

Another cross-sectional study was conducted among men exposed to 2-ethoxyethanol 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 fabrication (fab) workers compared with non-fabrication 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 employees) 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 non-significant 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.

Animal data:

Fertility impairment

There is one study available, designated as fertility assessment by continuous breeding (Lamb et al., 1984), 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 matings 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 was confirmed by 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 effect level (NOEL) for both sexes of about 0.5% (according to approximately 800 mg/kg bw/day) when applied via drinking water.

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 5.6 and the essential results of which are compiled in Table 5.9.1.B.

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

Table 8: Compilation of data of effects of 2-ethoxyethanol on the male reproductive system

Species	Protocol	Results
Inhalation 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 seminiferous 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 oedema, 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: not investigated for spermatotoxic effects (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 oedema, 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 ↓, 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 ↓, leucopoenia 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 oedema, absence of more mature sperm cells 100 µL/kg bw/day: NOEL (= 93 mg/kg/d) (Stenger et al. 1971)
Subcutaneous administration		
Rat (Wistar)	100, 200, 400 and 800 µL/kg bw/day 4 weeks (7d/week)	400 and 800 µL/kg bw/day: testicular oedema, absence of more mature sperm cells 200 µL/kg bw/day: NOEL (= 186 mg/kg/d) (Stenger et al. 1971)

Conclusion on fertility:

Human data from several epidemiological studies may indicate an association between exposure to 2-ethoxyethanol 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 observations from epidemiological studies in males appear plausible since testes toxicity was demonstrated in numerous studies in laboratory animals.

5.9.2 Developmental toxicity

Oral route of administration

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

In a study with Sprague-Dawley rats (Goad and Granmer, 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 embryo mortality was 31% of implants with no apparent effect on pup body weights. In 19 litters that received 270 to 400 mg/kg bw/day embryo mortality 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 embryo mortality 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. 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 3605 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 1000, 1800, 2600, 3400, and 4200 mg/kg bw/day. Dams were sacrificed at g.d. 18. Significantly reduced fetal body weight was revealed at the dose of 1000 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 (3400 mg/kg bw/day). An increased incidence of resorptions was observed at 1800 mg/kg bw/day associated with fewer live fetuses at termination. At the higher dose levels (3400 mg/kg bw/day) embryo mortality 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 1200 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 postnatal increasing mortality.

Inhalation 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 were observed also at 50 ppm (i.e. non-ossified 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. No effects on body weight gain or food consumption nor any clinical abnormalities were observed 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 was 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±31 ppm ("low level") or 617±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% embryo mortality as indicated by exclusively early resorptions in the uteri of all pregnant does. Also in the 160 ppm group the mean number of resorptions per litter and the number of litters with resorptions 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±18 ppm or 649±50 ppm, 7 hr/day, 5 days/week and/or 0 ppm, 202±11 ppm or 767± 2 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 embryo mortality 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 newborn 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.

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. 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 resorptions 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 or 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.

5.9.3 Human data

5.9.4 Other relevant information

Numerous *in vivo* and *in vitro* investigations have been demonstrating that the major toxic potential of both 2-ethoxyethanol is attributable to the 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 (Beatti and Brabec, 1986).

2-Ethoxyethanol tested in the embryonic stem cell test did not show an embroyotoxic potential (Verwei et al., 2006). The lack of metabolic capacity of stem cells could explain the negative result. Weak embryotoxic activity was identified for 2-ethxyacetic acid confirming that the metabolite accounts for the effects observed.

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 and Brightwell (1984) 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.

5.9.5 Summary and discussion of reproductive toxicity

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., 1984).

It is however evident from various other studies (c.f. Table 5.9.1.B) 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).

In addition studies with rabbits, rats and mice using the inhalation, 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 also be 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).

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

C&L, dose-response estimation including weight-of-evidence considerations.

5.10 Other effects

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not evaluated for this dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated for this dossier.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

2-Ethoxyethanol was an EU priority substance under the existing substance regulation (EEC) 793/93. The proposed (de-)classification (deletion of R21, harmful in contact with skin) was discussed and agreed at the TC C&L in September 2007.

Classification proposals were confirmed for the inhalation and oral route of acute toxicity and for reproductive toxicity (fertility impairment and developmental toxicity) and therefore these endpoints were the only endpoints adressed in this report. Repeated dose data were added for support of fertility data.

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