Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Methanol

EC number: 200-659-6
CAS number: 67-56-1

CLH-O-0000004421-84-03/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

12 September 2014

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON METHANOL

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: Methanol

EC Number: 200-659-6
CAS Number: 67-56-1

Index Number: 603-001-00-X

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Version number: 3 **Date:** October 2013

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Part A.

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Part A.

1. PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 SUBSTANCE

Type of substance methanol: Existing Chemical (composition); organic (origin). The characteristics and physico–chemical properties are described below (see the IUCLID dataset for further details).

Table 1: Substance identity

Substance name:	Methanol	
EC number:	200-659-6	
CAS number:	67-56-1	
Annex VI Index number:	603-001-00-X	
Degree of purity:	> 99.99 % (w/w)	
Impurities:	**Impurity**	**Typical concentration**

1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Flam. Liq. 2 H225 Acute Tox. 3(*) H331 Acute Tox. 3(*)H311 Acute Tox. 3(*)H301 STOT SE 1 H370 (**) Specific concentration limits STOT SE 1; H370: C ≥ 10 % STOT SE 2; H371: 3 % ≤ C < 10 %	F; R11 T; R23/24/25-39/23/24/25 Specific concentration limits T; R23/24/25: C ≥ 20% Xn; R20/21/22: 3% ≤ C < 20% T; R39/23/24/25: C ≥ 10% Xn; R68/20/21/22: 3% ≤ C < 10%
Current proposal for consideration by RAC	Repr. 1B – H360D	Repr. Cat. 2; R61
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Flam. Liq. 2 H225 Acute Tox. 3(*) H331 Acute Tox. 3(*)H311 Acute Tox. 3(*)H301 STOT SE 1 H370 (**) Repr. 1B – H360D Specific concentration limits STOT SE 1; H370: C ≥ 10 % STOT SE 2; H371: 3 % ≤ C < 10 %	F; R11 T; R23/24/25-39/23/24/25 Repr. Cat. 2; R61 Specific concentration limits T; R23/24/25: C ≥ 20% Xn; R20/21/22: 3% ≤ C < 20% T; R39/23/24/25: C ≥ 10% Xn; R68/20/21/22: 3% ≤ C < 10%

(*) Minimum classification

(**) The route of exposure should be indicated

1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	Flam. Liq. 2 H225	Not applicable	Flam. Liq. 2 H225	
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	Acute Tox. 3 (*) H301		Acute Tox. 3 (*) H301	
	Acute toxicity - dermal	Acute Tox. 3 (*) H311		Acute Tox. 3 (*) H311	
	Acute toxicity - inhalation	Acute Tox. 3 (*) H331		Acute Tox. 3 (*) H331	
3.2.	Skin corrosion / irritation	None		None	Not evaluated
3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	None		None	Not evaluated
3.5.	Germ cell mutagenicity	None		None	Not evaluated

3.6.	Carcinogenicity	None		None	Not evaluated
3.7.	Reproductive toxicity	Repr. 1B H360D		None	
3.8.	Specific target organ toxicity – single exposure	STOT SE 1 H370 (**)	STOT SE 1; H370: C ≥ 10 % STOT SE 2; H371: 3 % ≤ C < 10 %	STOT SE 1 H370 (**)	
3.9.	Specific target organ toxicity – repeated exposure	None		None	Not evaluated
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: *Danger*
Hazard statements: H225 H331 H311 H301 H370 H360D
Precautionary statements: not harmonised
Pictogram: GHS02 GHS06 GHS08

Proposed notes assigned to an entry: None

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	None		None	Not evaluated
Oxidising properties	None		None	Not evaluated
Flammability	F; R11		F; R11	
Other physico-chemical properties	None		None	Not evaluated
Thermal stability	None		None	Not evaluated
Acute toxicity	T; R23/24/25	T; R23/24/25: C ≥ 20 % Xn; R20/21/22: 3 % ≤ C < 20 % T; R39/23/24/25: C ≥ 10 % Xn; R68/20/21/22: 3 % ≤ C <10 %	T; R23/24/25	
Acute toxicity – irreversible damage after single exposure	T; R39/23/24/25	T; R23/24/25: C ≥ 20 % Xn; R20/21/22: 3 % ≤ C < 20 % T; R39/23/24/25: C ≥ 10 % Xn; R68/20/21/22: 3 % ≤ C <10 %	T; R39/23/24/25	
Repeated dose toxicity	None		None	Not evaluated
Irritation / Corrosion	None		None	Not evaluated
Sensitisation	None		None	Not evaluated
Carcinogenicity	None		None	Not evaluated
Mutagenicity – Genetic toxicity	None		None	Not evaluated
Toxicity to reproduction – fertility	None		None	The available data are not sufficient for classification
Toxicity to reproduction – development	T; R61		None	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	The available data are not sufficient for classification
Environment	None		None	Not evaluated

[1)] Including SCLs

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Classification

The substances classified:

•<u>for physical-chemical properties</u>:

F; R11 Highly flammable; Highly flammable

•<u>for health effects</u>:

T; R23/24/25Toxic; Toxic by inhalation, in contact with skin and if swallowed.
T; R39/23/24/25Toxic; Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed.
T; Repr Cat. 2 ; R61 May cause harm to the unborn child.

Labelling

Indication of danger:

F- highly flammable
T–toxic

R-phrases:

R11 - highly flammable.
R23/24/25 –toxic by inhalation, in contact with skin and if swallowed.
R39/23/24/25- toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
R61- may cause harm to the unborn child.

S-phrases:

S1/2 – keep locked up and out of reach of children
S7– keep container tightly closed
S16 -keep away from sources of ignition –No smoking
S36/37 – wear suitable protective clothing and gloves
S45 – in case of accident or if you feel unwell, seek medical advice immediately
(show the label where possible)
S53Avoid exposure - Obtain special instructions before use

Specific concentration limits:

Concentration	Classification
C ≥ 20 %	T; R23/24/25- T; R39/23/24/25
10 % ≤ C < 20 %	Xn; R20/21/22- T; R39/23/24/25
3 % ≤ C < 10 %	Xn; R20/21/22- Xn; R68/20/21/22

2 BACKGROUND TO THE CLH PROPOSAL

2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The classification of aqueous solutions of methanol is harmonised in Annex VI of CLP under the index number 603-001-00-X as follows:
Flam. Liq. 2 H225
Acute Tox. 3(*) H331
Acute Tox. 3(*) H311
Acute Tox. 3(*) H301
STOT SE 1 H370 (**)

Specific concentration limits
STOT SE 1; H370: $C \geq 10$ %
STOT SE 2; H371: 3 % $\leq C < 10$ %

2.2 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL

In general, prenatal developmental toxicity was evidenced by decreased foetal weight, decreased incidence of live foetuses and increased incidences of resorptions, dead foetuses, exencephaly, neural tube defects, cleft palate and skeletal and visceral malformations.

Based on animal studies, development is severely impacted in several species (rats, mice, rabbits and monkeys).

The Italian Competent Authority (IT-CA) considers that the current classification of methanol needs to be revised following the evaluation of the available data on toxicity to reproduction.

In 2010 the Committee of the Health Council of the Netherlands for Compounds toxic to reproduction has extensively evaluated all the available information on toxicity to reproduction for methanol. The final conclusion was: "In view of the data concerning prenatal developmental toxicity in experimental animals, the committee recommends classifying methanol in category 2 (*substances which should be regarded as if they cause developmental toxicity in humans*) and labelling methanol with T; R61 (*may cause harm to the unborn child*)".

Italy agrees with this conclusion, and presents a proposal for a revised harmonized classification according to article 36 of CLP.

A classification Repr.1B – H360D is proposed in the CLP regulation (Repr. Cat 2-R61 according to directive 67/548/EEC).

Performing the evaluation of data, IT-CA has moreover taken into account the information provided by the Registrant in his Registration dossier(IT-CA has taken into account all the bibliographic sources reported in the Registrant CSR and when the results of a previous study are included in a more recent publication, only the last one has been reported: eg Rogers et al. 1997 has been reported to consider even Rogers et al. 1993), the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Methanol (2003) and the OECD SIDS Initial

Assessment Report of Methanol (2004). Information on reproductive toxicity (both in experimental animals and in humans) considered in this report was collected by a literature search performed on EMBASE, MEDLINE, CAPLUS, BIOSIS, TOXCENTER, up to March 2013.

2.3 CURRENT HARMONISED CLASSIFICATION AND LABELLING

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

The classification of Methanol is harmonised in Annex VI of CLP under the index number 603-001-00-X as follows:

Table 3.1 (CLP)
Flam. Liq. 2 H225 Acute Tox. 3(*) H331 Acute Tox. 3(*)H311 Acute Tox. 3(*)H301 STOT SE 1 H370 (**) Specific concentration limits STOT SE 1; H370: C ≥ 10 % STOT SE 2; H371: 3 % ≤ C < 10 %

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

The classification of Methanol is harmonised in Annex VI of CLP under the index number 603-001-00-X as follows:

Table 3.2 (67/548/EEC)
F; R11 T; R23/24/25-39/23/24/25 Specific concentration limits T; R23/24/25: C ≥ 20% Xn; R20/21/22: 3% ≤ C < 20% T; R39/23/24/25: C ≥ 10% Xn; R68/20/21/22: 3% ≤ C < 10%

2.4 CURRENT SELF-CLASSIFICATIONAND LABELLING

Not relevant.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No justification is needed.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

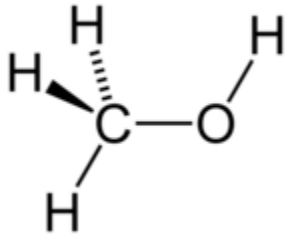
1 IDENTITY OF THE SUBSTANCE

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE

Table 5: Substance identity

EC number:	200-659-6
EC name:	Methanol
EC inventory:	200-659-6
CAS number:	67-56-1
CAS name:	Methanol
IUPAC name:	Methanol
CLP Annex VI Index number:	603-001-00-X
Molecular formula:	CH_4O
Molecular weight range:	32.0419

Structural formula:



1.2 COMPOSITION OF THE SUBSTANCE

Name: Methanol

Description: substance composition of methanol

Degree of purity: > 99.99 % (w/w)

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Methanol EC no.: 200-659-6	>= 99.99% (w/w)		

Table 7: Impurities

Not relevant for the classification.

1.2.1 Composition of test material

Relevant information could be extracted from the IUCLID 5 dossier in the respective studies when available.

1.3 PHYSICO-CHEMICAL PROPERTIES

Methanol is a colorless, flammable liquid with slightly alcoholic odor, completely miscible with water and organic solvents and is very hygroscopic. It is the simplest of a long series of organic compounds called alcohols. It can be made by reacting hydrogen with carbon monoxide or carbon dioxide in the presence of a catalyst at elevated temperatures and pressures. It is possible to produce Methanol by fermenting biomass and it has therefore also been called wood alcohol. Methanol is a common industrial solvent and chemical intermediate in the production of *t*-butyl methyl ether, glycol ethers.

Table 8: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	liquid	HSDB 2007	
Melting/freezing point	-97.8 °C	HSDB 2007	
Boiling point	64.7°C	HSDB 2007	
Relative density	0.79 to 0.8 Relative density D20/4	Beilstein 2007	
Vapour pressure	169.27 hPa at 25°C	HSDB 2007	
Surface tension	no surface activity Based on chemical structure, no surface activity is predicted	Expert judgement	
Water solubility	Miscible Substance is completely miscible in water at 20°C	HSDB 2007	
Partition coefficient n-octanol/water	- 0.77	Beilstein 2007	
Flash point	9.7°Cat 1013hPa	See confidential version (IUCLID file)	
Flammability	Highly flammable liquid The substance has no pyrophoric properties and does not liberate flammable gases on contact with water. The flammability is deduced from flash point and boiling point, so the substance is a highly flammable liquid	Expert judgement	
Explosive properties	Non explosive There are no chemical groups associated with explosive properties present in the molecule	Expert judgement	
Self-ignition temperature	455°Cat 1013hPa	See confidential version (IUCLID file)	
Oxidising properties	No oxidising properties. Substance is incapable of reacting exothermically with combustible materials	Expert judgement	
Granulometry	Not applicable. Substance is marketed or used in a not solid or granular form	Expert judgement	
Stability in organic solvents and identity of relevant degradation products	Not applicable. The stability of the substance is not considered as critical	Expert judgement	
Dissociation constant	Not applicable. The substance does not contain any ionic	Expert judgement	

	structure under environmental conditions		
Viscosity	0.544- 0.59 mPas at 25°C	Beilstein 2007	

2 MANUFACTURE AND USES

2.1 MANUFACTURE

Manufacturing process

The methanol production process converts a gaseous mixture of carbon oxides and hydrogen, derived in a steam reforming of a hydrocarbon feedstock, typically natural gas, into methanol. This mixture is compressed and then reacted over a metal oxide catalyst to give methanol and by-products, according to the following reactions.

$CO + 2\ H_2 <-> CH_3OH$
$CO_2 + 3\ H_2 <-> CH_3OH + H_2O$.
The pure product is obtained by fractional distillation. All process steps are performed in closed systems.

2.2 IDENTIFIED USES

Methanol is used in a variety of industrial applications. The primary use for methanol is as a fuel. It is also used for waste water treatment and for producing biodiesel.
Methanol is used in the production of formaldehyde, acetic acid, chloromethanes, methyl methacrylate, methylamines, dimethyl terephthalate, and as a solvent or antifreeze in paint strippers, aerosol spray paints, wall paints, carburetor cleaners, and car windshield washer compounds.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

In mammalian methanol (MeOH) is readily absorbed after inhalation, ingestion and dermal contact and distributes rapidly throughout the body. Metabolism in humans, rodents, and monkeys contributes up to 98 percent of the clearance, with more than 90 percent of the administered dose exhaled as carbon dioxide (CO_2). Renal and pulmonary excretion contributes only about 2 – 3 percent. The metabolism and toxicokinetics of MeOH varies by species and dose. In humans, the half-life time is approximately 2.5 – 3 hours at doses lower than 100 mg/kg bw. At higher doses, the half-life can be 24 hours or more (IPCS/WHO, 1997; Kavet and Nauss, 1990).

The metabolism of MeOH occurs mainly in the liver, where MeOH is initially converted to formaldehyde, which is in turn converted to formate. through a series of oxidation steps to sequentially form formaldehyde, formate, and CO_2 (Figure 1).

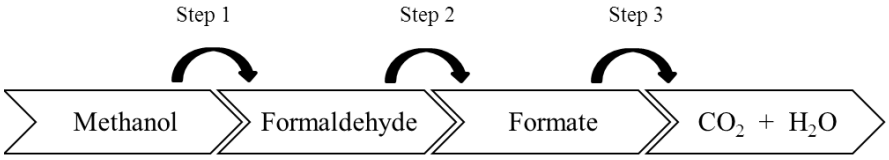


Figure 1.: the mammalian metabolism of MeOH

Step 1.

The first step in the metabolic sequence is oxidized to formaldehyde.

In humans and monkeys, the conversion to formaldehyde is mediated by alcohol dehydrogenases (ADH) and CYP2E1 basically limited to the capacity of those enzymes.

In rodents, the oxidation to formaldehyde predominantly employs the catalase-peroxidase pathway and to a lesser extent by alcohol dehydrogenases (ADH1).

Rabbits, like humans, may largely use ADH to metabolize MeOH (as described by an in vitro study using hepatic homogenates by Otani, 1978 reported in Sweeting et al., 2010) and more accurately than rodent reflect primate MeOH and formic acid pharmacokinetic profiles (Sweeting et al., 2011; Sweeting et al., 2010).

In rodents, the rate-limiting step in the metabolism of MeOH is the oxidation of MeOH to formate, while the oxidation of formate to CO_2 is rate limiting in primates. As a consequence, exposure to high concentrations or doses of MeOH may cause accumulation of MeOH in rodents and of formate in primates. In humans, accumulation of formate may occur at MeOH doses >210 mg/kg bw (Kavet and Nauss, 1990).

Step 2.

The second metabolic step converts formaldehyde to formic acid, which, in turn, dissociates to formate and a hydrogen ion.

In all species, formaldehyde is rapidly converted to formate (half-life ~1 minute), and does not accumulate in animals or humans exposed to MeOH.

Formaldehyde is oxidized to formate by two metabolic pathways (Teng et al., 2001).

The first pathway involves conversion of free formaldehyde to formate by the so-called low-affinity pathway (affinity = 1/KM= 0.002/μM) mitochondrial aldehyde dehydrogenase-2 (ALDH2). The second pathway involves a two-enzyme system that converts glutathione-conjugated formaldehyde (S-hydroxymethylglutathione (HMGSH)) to the intermediate S-formylglutathione, which is subsequently metabolized to formate and glutathione (GSH) by S-formylglutathione hydrolase. The first enzyme in this pathway, formaldehyde dehydrogenase-3 (ADH3), is rate limiting, and the affinity of HMGSH for ADH3 (affinity = $1/K_m$ = 0.15/μM) is about a 100-fold higher than that of free formaldehyde for ALDH2. In addition to the requirement of GSH for ADH3 activity, oxidation by ADH3 is nicotinamide adenine dinucleotide- (NAD^+-)dependent (see Figure 2).

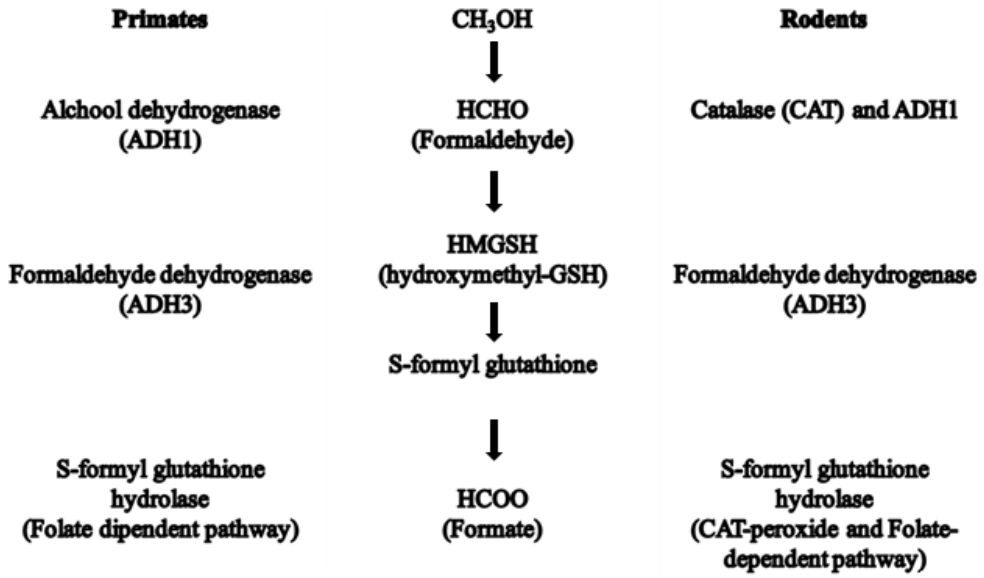


Figure 2: the metabolic pathway of MeOH (Source: IPCS, 1997)

Under normal physiological conditions NAD^+ levels are about two orders of magnitude higher than NADH, and intracellular GSH levels (mM range) are often high enough to rapidly scavenge formaldehyde (Svensson et al., 1999); thus, the oxidation of HMGSH is favorable. In addition, genetic ablation of ADH3 results in increased formaldehyde toxicity (Deltour et al., 1999). These

data indicate that ADH3 is likely to be the predominant enzyme responsible for formaldehyde oxidation at physiologically relevant concentrations, whereas ALDHs likely contribute to formaldehyde elimination at higher concentrations (Dicker and Cedebaum, 1986).

Step 3

The last reaction step in the MEOH metabolism is the conversion of formate to CO_2 (and H_2O) by the formyl-tetrahydrofolate synthetase. In this step, formate combines with tetrahydrofolic acid (THF) to form 10-formyl-THF through the action of formyl-THF synthetase. Next, 10-formyl-THF is converted to CO_2 by formyl-THF dehydrogenase.

Rodents convert formate to CO_2 through a folate-dependent enzyme system and a CAT-peroxide system. Formate generates CO_2^- radicals, and can be metabolized to CO_2 via CAT and via the oxidation of N^{10}-formyl-THF. Unlike rodents, formate metabolism in primates occurs solely through a folate-dependent pathway. Black et al. (1985) reported that hepatic THF levels in monkeys are 60% of that in rats, and that primates are far less efficient in clearing formate than are rats. Formic acid and MeOH have common mechanisms of toxicity, because formic acid is a metabolic end product of MeOH and is mainly responsible for the toxic inhibition of cytochrome c oxidase. Inhibition of the cytochrome c oxidase complex leads to anaerobic glycolysis and lactic acidosis (‘‘histotoxic hypoxia’’) (Dikalova et al., 2001).

In a study in which a comparison of formate elimination in wild type and FDH-deficient (NEUT2) mice after formate application it was determined that the oxidation of formate by the folate-depent FDH (FDH: 10-formyltetrahydrofolate dehydrogenase, which catalyzes the oxidation of excess folate-linked one-carbon unit) was predominat at low formate levels, but was not apparent at high formate levels.

This doesn’t happen when the catalase (CAT) was inactivated by treatment with 3-aminotriazole (a CAT inhibitor). These results indicate that mice may have three or more systems capable of oxidizing formate: FDH is predominant pathway at physiological levels, CAT at high levels, and a third or more undefined systems appear to function at both low and high format levels. In addition primates do not appear to exhibit such capacity and are more sensitive to metabolic acidosis following MeOH poisoning (Cook et al., 2001).

Formaldehyde as toxic metabolite of MeOH

The cytotoxicity of formaldehyde was clearly related to its metabolism. Inhibition of ADH1, ALDH2 and ADH3 were found to inhibit the removal of formaldehyde by the hepatocytes, which resulted in increased cytotoxicity through oxidative stress mechanisms. It is reasonable to hypothesise that individuals with deficiencies in any of the above enzymes as well as those who have lower levels of GSH will be more susceptible to formaldehyde toxicity. Such individuals are likely to include approximately 50% of Orientals, who possess a mutant, inactive ALDH2, as well as diabetics, who already have carbonyl glycoxidative stress as a result of aldehyde accumulation. In addition, it has been shown that the activity of ALDH2 is partially hormonally regulated in that

high levels of female hormones such as estrogen and progesterone can down-regulate ALDH2. Thus, women who are pregnant or are taking oral contraceptives may be more susceptible to HCHO. Although HCHO is indeed rapidly removed in the healthy individual, extra caution must be taken by those who lack any part of the formaldehyde cellular defence system (Teng et al., 2001).

Formic acid as toxic metabolite of MeOH

Formic acid is a toxic metabolite of MeOH in mammals, leading to acidosis. Formic acid accumulation occurs in human, rabbit and primates but not in rodents and leading to a disproportionate increase of formate in the blood and in sensitive target tissues such as Central Nervous System and the retina.

Primates naturally have lower folate concentrations than do rodents they have considerably less capacity to metabolize formate (Johlin et al., 1987). The result is that primates may accumulate levels of formate that exert toxicological consequences at doses far lower than those needed to produce equivalent effects in rodents. In addition several factors predispose humans to folate deficiencies or decreases in folate activity from MeOH. (Medinsky et al., 1997; Dorman et al., 1994; Medinsky and Dorman, 1995)

Potentially Sensitive Sub-populations

Each of the enzymes involved in MeOH metabolism (ADH, ALDH, and CYP2E1) exists as a family of isoenzymes. Individual, gender, age and specie variations in the quantity of these isoenzymes influence several factors such as the rate of MeOH clearance from the blood, and differences in individual susceptibility (Sweeting et al., 2010).

Population studies reveal significant ethnic differences in these genes with greater ethanol susceptibility in Asian and Native American populations. Given that MeOH metabolism in humans is similar to ethanol, these polymorphisms in the alcohol dehydrogenase allele may lead to greater susceptibility to MeOH toxicity. This would result from decreases in metabolism leading to higher peak-blood levels.

4.2 Acute toxicity

Not evaluated in this dossier.

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

Not evaluated in this dossier.

4.8 Specific target organ toxicity (CLP regulation)- Repeated exposure (STOT RE)

Not evaluated in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Not evaluated in this dossier.

4.11.1.1 Non-human information

Not evaluated in this dossier.

4.11.1.2 Human information

Not evaluated in this dossier.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 1: Summary table of developmental toxicity oral, I.V. and I.P. studies.

Method	Results	Remarks	Reference
New Zealand Rabbits I.P. Dosing for teratology studies: Rabbits: two doses of 2 g/kg bw GD 7 or 8 Rabbits sacrificed on GD 29	No effects on maternal toxicity was reported. No effects on the incidence of fetal resorptions, stillbirth or postpartum lethality. No effect on fetal body weights. MeOH caused a 4.4 fold increase in tail abnormalities (including short tails and absent tails). In addition several other malformations were observed in treated litters: open posterior neuropore in addition to tail abnormalities (2 foetuses in one litter), abdominal wall defect (one foetus), frontal nasal hypoplasia (3 foetuses).	Experimental results 2 (reliable with restrictions) Weight of evidence Test material: MeOH	Sweeting et al., 2011
CD-1 mice gavage Doses: 0, 4.0 and 5.0 g/kg bw GD 7	Dams No effects Foetuses Foetal weight and the incidences of live and dead foetuses were not affected. The numer of resorptions shows an increase between doses (1.3, 4.3 and 6.0 for 0, 4.0 and 5.0 g/kg bw). Skeletal examinations revealed that maternal MeOH exposure can alter segment patterning in the developing mouse embryo, resulting in posteriorisation of cervical vertebrae. Rib on C7: 0, 10 and 28** %; Tubercula anterior on C5: 1, 10 and 30**%; Split and/or fused C1: 0, 3 and 10 %; Split and/or fused C2: 8, 8 and 41** %; 25 presacral vertebrae: 2, 5 and 10 %; > 7 attached ribs: 0, 30* and 28* %; Offset sternebrae: 3, 25** and 22** %; Clef palate: 0, 19** and 14 % The values are referred to foetus/total foetus % * different from control p≤ 0.05 ** different from control p≤ 0.01	Experimental results 2 (reliable with restrictions) Weight of evidence Test material: MeOH	Connelly and Rogers, 1997
Long-Evans rats gavage Doses: 0, 1.3, 2.6 and 5.2 ml/ kg bw GD 10	Dams 5.2 ml/ kg bw Body weight and food consumption were statistically decreased. Foetuses At all dose levels foetal body weights were statistically significantly decreased, (no dose	Experimental results 2 (reliable with restrictions) Weight of evidence Test material: Methanol	Youssef et al, 1997

	relationship was observed). Incidence of foetuses showing anomalies and/or variation (undiscended testes, exophthalmia and anophthalmia) was statistically significant increased. Total foetuses with anomalies: 1/06, 5/3.7*, 9/7* and 22/16.5* % Undiscended testes: 0/0, 1/07, 3/2.3 and 12/9*%; Exophthalmia and anophthalmia: 0/0, 0/0, 3/2.3 and 10/7.5* %; Total foetuses with anomalies and/or variations: 23/14, 45/33*, 52/41* and 79/59*%. The values are referred to foetus/foetus % * different from control $p \leq 0.05$		
CD-1 mice Dams Gavage Doses: For MeOH 0 and 5.0 g/kg bw GD 6-10 For Folic acid diet 400 (marginal), or 1,200 (control) nmol folic acid/kg diet during the entire study, and 1% of succinylsulphatyazole (starting 5 weeks prior to mating) Sacrificed at GD18	Net maternal weight gain was not affected by dietary folic acid or MeOH treatment. Maternal body weights were similar among the groups throughout gestation with the exception that on GD 18, dams fed adequate folic acid and treated with water had higher body weights than the marginal folic acid-water group. Non-gravid maternal body weights were similar among the groups. Implantation sites, live and dead foetuses, and resorptions were counted; foetuses were weighed individually and examined for cleft palate and exencephaly. The marginal folic acid dietary treatment resulted in low maternal liver (50% reduction) and red cell folate (30% reduction) concentrations, as well as low fetal tissue folate concentrations (60 to 70% reduction) relative to the adequate folic acid dietary groups. Marginal folic acid treatment alone resulted in cleft palate in 13% of the litters; there were no litters affected with cleft palate in the adequate folic acid - control group. Marginal folic acid -MeOH treatment resulted in a further increase in the litters affected by cleft palate (72% of litters affected). The percent of litters affected by exencephaly was highest in the marginal folic acid -MeOH group. These results show that marginal folate deficiency in pregnant dams significantly increases the teratogenicity of MeOH.	Experimental results 2 (reliable with restrictions) Supporting study Test material: MeOH and Folic Acid	Fu S.S. et al., 1996
Mice: CD1 (dams) gavage Exposure regime: For MeOH: -0, 4.0 and 5.0 g/kg bw GD 6-15 For Folic acid diet	During gestation, maternal body weights were significantly affected by dietary folic acid treatment. Dams in the 400 nmol/kg group had significantly lower body weights compared to dams in the 600 and 1.200 nmol/kg groups. MeOH significantly reduced the gestational weight gain in dams fed the 600 and 1,200 nmol/kg diets.	Experimental results 2 (reliable with restrictions) Supporting study Test material: MeOH and Folic Acid	Sakanashi et al., 1996

-400 (low), 600 (marginal), or 1,200 (adequate) nmol folic acid/kg diet during the entire study, (starting 5 weeks prior to mating) Sacrificed at GD18	Both of these parameters were affected by folate treatment; dams in the 400 nmol/kg folate group gained less weight compared to the 600 and 1.200 nmol/kg groups. MeOH did not affect these parameters. Maternal hematocrit levels were not affected by either MeOH or folate treatment. Plasma folate concentrations were not significantly affected by folate or MeOH treatment. Maternal liver weight was increased with low dietary folate; MeOH treatment resulted in an increase in liver weight in the 600 nmol/kg folate group. However, when based on non-gravid body weight, only folate treatment had an effect. Similarly, kidney weights were increased with the lower diet folate and MeOH treatment. Relative kidney weights based on non-gravid body weights were affected only by folate treatment. There was no effect of either treatment on total or relative spleen weight. Gravid uterus weights were lowest in the low dietary folate and MeOH groups with the lowest value occurring in the 400 nmol/kg group treated with the 5 g/kg bw methanol dose. This lower gravid uterus weight reflected an increased number of resorptions in the low folic acid and methanol treated groups. Foetuses were examined for external (cleft palate and exencephaly) and skeletal anomalies. Both MeOH and low dietary folic acid increased the incidence of cleft palate, with the highest number of affected litters in the low dietary folic acid group. These results support the concept that maternal folate status can modulate the developmental toxicity of methanol. In conclusion, both MeOH and low dietary folic acid increased the incidence of cleft palate, with the highest number of affected litter in the low dietary folic acid group. These results support the concept that the maternal folate status can modulate the developmental toxicity of MeOH.		
Pregnant rat Sprague-Dawley and mouse CD-1 Intrauterine microdialysis study MeOH exposure : - i.v. bolus 100 and 500 mg/kg bw - infusion 100 and 1000 mg/kg hr ^{3}H2O administration: 20 µCi/kg on GD 14 and 20 rats and GD 18 mice	Rats: - GD 20, initial $^{3}H_2O$ uptake rate was decreased 31% by a 100 mg/kg methanol dose and 45% by a 500 mg/kg dose - at GD 14 the $^{3}H_2O$ uptake rate was decreased by 30 and 57% for the 100 and 500 mg/kg doses, respectively. Mice: - initial uptake rate was decreased 26 % with the 100 mg/kg methanol bolus to the dam and 47% with the 500 mg/kg bolus. These data indicate that methanol may decrease uteroplacental blood flow, decreasing methanol presentation to the conceptus and possibly producing conceptal hypoxia.	Experimental result 2 (reliable with restrictions) Supporting study Test material: MeOH	Ward and Pollack (1996).

Wistar rats gavage Doses: 0, 2.5 g/kg body weight/day GD 6-15	No effects on maternal toxicity was reported Foetuses Foetal weight was statistically significantly, decreased. The incidence of foetuses showing skeletal anomalies, particularly extra cervical ribs, was statistically significantly increased. Fetal weight: 4.6±0,6 and 4.3±0.4* %; % of foetus with skeletal anomalies: 6 and 45*; Ribs 3 and 36* %; cervical (extra): 1 and 35* %. * different from control p≤ 0.05	Experimental results 2 (reliable with restrictions) Supporting study Test material: MeOH	De-Carvalho et al., 1994
Long-Evans rats drinking water Doses: 2% MeOH (about 2.5 g/kg body eight/day two group at the same concentration). GD 15-17 GD 17-19	No effects on maternal toxicity was reported. No effects were observed on litter size, pup mortality, birth weight, pup weight gain during lactation and the day of eye opening. Pups The proportion of pups successfully attaching to nipples did not differ significantly across the treatment groups ($F(2,27) = 2.35$). The methanol groups significantly from control group latencies ($F,(2,27) = 7.57$, $P < .01$). Prenatal exposure to methanol, therefore, produced a significant impairment in suckling behaviour that was evident 24 hours after birth. The proportion of pups successfully reaching the home area within 3 minutes did not differ across treatment groups, ($F(2,27) = 2.16$). On the other measures of homing behaviour, the methanol groups were quite similar, and both differed sharply from the control group. Of pups that successfully reached the home area, those exposed prenatally to methanol exhibited significantly longer latencies than controls ($F(2,27) = 23.01$, $P < .001$). The methanol-exposed animals took about twice as long as control pups. Their increased latencies may have been due, in part, to the tendency for methanol-exposed pups to choose the wrong initial direction more often than controls. Further, pups in both methanol groups crossed significantly more rectangles than controls to reach the home area ($F(2,27) = 11.34$, $P < .01$). In addition, the total number of rectangles crossed during the entire homing test was significantly elevated over control levels ($F(2,27) = 7.19$, $P < .01$).	Experimental results 2 (reliable with restrictions) Weight of evidence Test material: MeOH	Infurna R. and Weiss B., 1986

Table 2: Summary table of developmental toxicity inhalation studies

Method	Results	Remarks	Reference
Monkeys Macaca fascicularis The two-cohort study design used 48 adult female Macaca fascicularis (24/cohort) monkeys exposed whole body to 0, 200, 600, or 1800 ppm MeOH vapor for approximately 2.5 h/day, 7 days/week prior to breeding and throughout pregnancy.	Dams Although not statistically significant, five MeOH-exposed females were C-sectioned due to pregnancy complications such as uterine bleeding and prolonged unproductive labor. The mean length of pregnancy in the MeOH-exposed groups was significantly decreased by 6 to 8 days when compared to controls. Pups There were no MeOH-related effects on offspring birth weight or newborn health status. A total of 34 live-born infants were delivered (control=8, 200 ppm=9, 600 ppm=8, 1800 ppm=9). One female each in the control and 600-ppm group delivered a stillborn infant and a cesarean section (C-section) was required to deliver a hydrocephalic infant who died in utero in the maternal 1800-ppm group. Overall results: the results of the present study indicate that, for this nonhuman primate model, daily 2.5 h exposures to MeOH vapor from 200 to 1800 ppm for nearly 1year do not cause overt maternal toxicity in M. fascicularis females. The menstrual cycle and the ability of females to conceive were unaffected by these exposures. The incidence of maternal complication during pregnancy and delivery was high in the MeOH-exposed females (28% (8/28), for the MeOH exposed females versus 22% (2/9) for the control). The increase in complications however, was not statistically significant when compared to controls. The health status of live-born offspring was unaffected by maternal MeOH exposure. MeOH exposures were associated, however, with a reduction in the length of pregnancy (168, 160, 162 and 162 days). The reduced pregnancy lengths of the MeOH-exposed females may reflect the premature activation of the fetal HPA axis that controls timing of birth. Whether this represents a direct (fetal) or indirect maternal treatment effect is unknown. Independent of the specific biological mechanism, the reduced pregnancy durations of MeOH-exposed dams suggest a systematic disturbance in the timing of labor and delivery	Experimental result 2 (reliable with restrictions) Weight of evidence Test material: MeOH	Burbacher et al., 2004
Monkeys Macaca fascicularis Concentrations: 0 (n=11), 200 (n=12), 600 (n=11) and 1800 (n=12) ppm (0, 262, 786, 2358	No effects on maternal toxicity was reported Pups Weight and size: No effects were observed of the infants at birth and at nine month of age (severe wasting, resulting in euthanasia, was observed in two female pups of the high dose group	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: MeOH	Burbacher et al., 1999

mg/m3, respectively) for 2.5 h/day, Observation period: days/week during premating (about 120 days), mating (about 65 days) and gestation (about 163 days)* and daily until postnatal (PN) day 147, and then weekly. *The study was originally designed as a fertility study.	after 12 months of age). Neurobehavioural function tests did not show significant MeOH-related effects on most domains of early behavioural development. No effects on social and neuro/behavioural development. However, MeOH exposure was associated with a delay in early sensorimotor development for male infants of all dose groups and with deficits in visual recognition memory for all infants of all dose groups.		
Crl and CD-1 mice Concentrations: 0 or 10000 ppm (0 and 13100 mg/m^3) GD 6-7 for 7 h/day GD 7-8 for 7 h/day GD 8-9 for 7 h/day GD 9-10 for 7 h/day GD 10-11 for 7 h/day GD 11-12 for 7 h/day GD 12-13 for 7 h/day or to single day (7 hour) exposures during GD 5, 6, 7, 8 and 9. Number of litters: 12 – 14 for most critical period. Equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	Dams Peak maternal blood MeOH concentration at the end of the exposure was about 4 mg/mL, MeOH was cleared from maternal blood within 24 hr. Some fully resorbed litters were observed with 2-day MeOH exposure. Litters GD 6-7 Fetal weight was decreased as compared to their controls (1.10 and 0.97 g.). Number of dead and resorbed foetuses was increased (0.2 and 3.3* %). GD 7-8. Number of dead and resorbed foetuses was increased (0.8 and 2.9* %). GD 10-11 Number of live foetuses per litter was decreased (12.3 and 8.1* %) Foetuses (two-days exposure): Significantly increased of incidences compared to controls for 2 day exposure: cleft palate, exencephaly and skeletal defects were the fetal anomalies observed. - Cleft palate: occurred with 2-day exposures on GD 6-7 through GD 11-12 (peak on GD 7-8) and with 1-day exposures on GD 5 through 9 (peak on gd 7); - Exencephaly: occurred with 2-day exposures on GD 6-7 through GD 8-9 (peak on GD 6-7) and with 1-day exposure on GD 5 through 8 (peak on GD 7); - Skeletal elements malformed included the exoccipital (peak on GD 6-7 (22.5 %); GD 5 (9.9%)), atlas (peak on GD 6-7 (72.3 %); GD 5, 6 (55.5 %, 55.3 %)), axis (peak on GD 6-7(22.3 %); GD 7 (28.8 %)), cervical vertebra 7 with a rib (peak on GD 6-7 (73.7 %); GD 7 (45.4 %)) and lumbar vertebra 1 with a rib (peak on GD 7-8 (68.3 %); GD 7 (39.4 %). Foetuses (1-day exposure): An increase incidence of foetuses with 25 presacral vertebrae (normal 26) was observed with MeOH exposure on GD 5; whereas an increased incidence of foetuses with 27 presacral vertebrae was observed with methanol exposure on GD 7. According to the authors the results of this study indicate that gastrulation and early organogenesis represent the period of	Experimental result 2 (reliable with restrictions). Weight of evidence Test material: MeOH	Rogers and Mole, 1997

	increased embryonic sensitivity to MeOH. * different from control p≤ 0.05		
Rats (Long–Evans) Concentrations: 4500 ppm (5895 mg/m^3) GD 6 until PN day 21 for 6 h/day.	Dams No effects on body weight. Subtle behavioral changes were observed. Pups Subtle behavioral changes were observed. No effect on body weights was observed.	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: MeOH	Stern et al, 1997
Mice CD-1 Concentrations: 0 or 10,000 ppm (0 or 13100 mg/m^3) GD:8 for 6h/day	Inhalatory MeOH exposure induced signs of acute MeOH toxicosis (central nervous system depression and ataxia) which resolved within 1 h after the end of the exposure period. The incidence of open anterior neural tubes in GD 10 embryos (0.0 and 9.65 ± 3.13* %) was statistically significantly increased. * different from control p≤ 0.05	Experimental result 2 (reliable with restrictions). Weight of evidence Test material: MeOH	Dorman et al. 1995
Rat (Long-Evans) Concentrations: 0 or 15,000 ppm (0 or 19650 mg/m3) GD:7-19 for 7h/day Observation in pre-natal and post-natal period (60 days)	Dams Body weights were decreased during the first days of exposure. Pups No treatment related effects were observed on pup mortality (2 dead pups at birth in control group). Incidence of malformed pups (two malformed pups in one litter of MeOH-treated group showing anophthalmia and agenesis of optical nerve), litter size (10.8 vs 10.2) and implantation loss (13.8 vs 11.8) but on PN day 1 (7.1 vs 6.4*g) and 35 (females/males 122/139 g and 116/129** g) pup weights were slightly, but statistically significantly, lower in the MeOH treated animals than in the control animals. Except for a small delay in vaginal opening (29.7 vs 31.4** day), no effects were observed on any of the developmental parameters measured. * different from control p≤ 0.05 ** different from control p≤ 0.01	Experimental result 3 (not reliable). Supporting study Test material: MeOH	Stanton et al. 1995
Mice (CD-1 ICR BR) Concentrations: 0-10.000 ppm (0-13.100mg/m^3) GD:6-15 for 6h/day GD:7-9 for 6h/day GD:9-11 for 6h/day Pilot study	No effects on maternal toxicity was reported. Foetuses: GD at 6-15 for 6h/day Reduced foetal body weights (0.93±0.02 and 0.810.03* g) and increased incidences of resorptions (4.4 and 32.2* %), neural tube defects (0 and 46*%) , cleft palate (0 and 82 %) and digit malformations were observed /(0 and 36* %). GD at 7-9 for 6h/day The incidence of resorptions (1.1 and 13.4*%), neural tube defects (0 and 33%) and cleft palate (0 and 33%) , but not the incidence of digit malformations, was increased whereas the number of live foetuses was decreased (12.8±0.5 and 10.4±0.9* %). GD at 9-11 for 6h/day Only cleft palate (0 and 24*%) and digit malformations (0 and 12) but no neural tube	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: Methanol	Bolon et al., 1993

	defects were observed. * different from control p≤ 0.05		
Mice (CD-1 ICR BR) Concentrations: 0, 5000, 10000 and 15000 ppm (0, 6550, 13100 and 19650 mg/m3) GD:7-9 for 6h/day 15,000 ppm (19650 mg/m3) GD:9-11 for 6h/day GD:7 for 6h/day 15,000 ppm (19650 mg/m3) GD:7, 8 or 9 for 6h/day GD:7, 8 or 8,9 for 6h/day	Dams GD:7-9 for 6h/day: At 15,000 ppm maternal body weight gain during gestation was decreased and neurological symptoms (ataxia, circling, tilted heads or depressed motor activity) were observed on the first days of exposure. The number of resorptions was increased in all groups (2.7, a 0.5, 16.6 and 46.2* %). Foetal: 15,000 ppm GD:7-9 for 6h/day: the number of live foetuses (12±0.4 of the control group vs 7.9±1.1* %), and foetal weight were statistically significantly decrease (0.92±0.05 of the control group vs 0.82±0.02* %). Developmental effects, 7-9, 0 5.000, 10.000 and 15.000 ppm: - neural tube defects: 0, 0, 30 and 65* %; - cleft palate: 9, 4, 50* and 88 %; - renal variations: 41, 100*, 90 and 75%; - ocular defects: 0, 0, 10* 53 %; - tail anomalies: 0, 0, 40* and 65%. Dams GD: 9-11 for 6h/day: The dams showed neurological symptoms but no effect on body weight and resorptions was observed. Foetal GD: 9-11 for 6h/day: No neural tube defects and ocular defects were observed while renal variations, cleft palate, and limb and tail anomalies were observed. Dams GD 7 No effects on maternal body weight Neurological effects (ataxia, circling, tilted heads or depressed motor activity) were observed. Resorptions were increased at 15.000 ppm (2.7 of the control group vs 39*%) as consequence the number of live foetus was decreased. * different from control p≤ 0.05	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: MeOH	Bolon et al, 1993
Rats (Sprague-Dawley) Concentrations: 0-10.000-20.000ppm (0-13.100-26.200mg/m3 GD:1-19 at 0-10,000 for 7h/day GD:7-15 at 20.000 for 7h/day 0-5000 ppm (0-6.550 mg m3) GD:1-19 at 0-10,000 for 7h/day	Dams: slight unsteady gait only during the first days of exposure no effects on the body weight and food consumption. Foetal: No resorptions 20.000 ppm dose: In total 93% of litters and 54% of foetuses were affected by: Statistically significant weight decrease (female/male control group: 3.15±0.32/3.34±0.36 vs	Experimental result 2 (reliable with restrictions). Weight of evidence Test material: Methanol	Nelson et al. 1985

	2.76*±0.47/2.82*±0.56 g.) Statistically significant increase in the incidence of skeletal malformations (0 in the control group vs 72 %) in cranium, vertebrae and ribs and visceral malformations (0 in the control group vs 15 % (in eye, brain-exencephaly and encephaloceles- and cardiovascular and urinary system). 10,000 ppm dose: Statistically significant weight decrease (female/male control group: 3.15±0.32/3.34±0.36 vs 2.93*±0.26/3.12*±0.30 g.), this effect may be caused by the increased number of foetuses. Increase in the incidence of skeletal malformations (0 in the control group vs 2 %) in cranium, vertebrae and ribs and visceral malformations (0 in the control group vs 2 %) in eye, brain-exencephaly and encephaloceles- and cardiovascular and urinary system even if not statistically significant. 5000 ppm dose: No adverse effects In conclusion it was observed that the % of litter with abnormal foetuses for 0, 5.000, 10.000 and 20.000 ppm was 0, 15, 47 and 93*%. Foetal NOEL: 5000 ppm Maternal NOAEL: 10000 ppm (as noted by NPT Expert Panel).		
Rats (Sprague-Dawley) Concentrations: 0-200-1000-5000(0-: 0.27, 1.33, 6.65 mg/L GD:1-17	Dams: 5000 ppm dose: decrease in body-weight gain, food and drinking water consumption. One dam died, another one had to be killed before delivery. After delivery: gestation time was prolonged (0.7 days); food and drinking water consumption were reduced during lactation; Foetal: 5000 ppm dose: About 50 % of the fetuses with ventricular septal defects (visceral malformation in 16/20 litters or 64/131 fetuses) vs. 0% or near 0% in all other groups, and residual thymus (variation in all 20 litter or 70/131 fetuses) vs. about 2.4 to 2.9 % in 4 litters each of all other groups. Other changes included significantly increased incidence of skeletal anomalies: "atresia of cervical arch/vertebra foramen costotransversarium" (45%), " bifurcated vertebral center" (14%) and "cervical rib" (65%) as well as "excessive sublingual neuropore" (50%), all of which malformations having no or little relevance in the other group except of "atresia foramen" with about 25 % in the control and about 4 to 8 % in the other exposure groups. Neo-/postnatal findings: live fetuses showing poor vitality (ca. 17% = on average 2/12 pups per litter died as compared with overall	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: Methanol.	Takeda K. and Katho N., 1988

	mortality 1 to 2% in the other groups). Retardation of growth was significantl up to at weaning. Water consumption was reduced, in particular for females. At 8 weeks, brain, thyroid (males), thymus and testis weights were lower ($p<0.01$), and pituitary-gland weight of males was higher ($p<0.05$); 16. % of the offsprings (15/91 in 8/12 litters) had hemilateral absence of thymus . Maternal/developmental NOAEC 1.33 mg/L – LOAEC 6.65 mg/L		
Rat (Sprague-Dawley) (two-generation study – OECD 416). Concentrations: 0-10-100-1000 ppm (0; 0.013; 0.13; 1.3 mg/L) Exposure F0: 103 -108 d F1: 61 -62 d and 145 - 153 d F2: 54 -56 d	F0: no effects were observed. F1: males pups 1.3 mg/L: testis descent was completed within 16 through 20 post-natal days with the maximum at day 17 and 18 (32 and 39%, respectively), while in the respective control, descent was complete from 16 through 21 days with the maximum at day 19 (32 %), indicating an earlier descent related to treatment. Absolute and relative brain weights were significantly lowered in the high-dose groups of either sex at an age of 8 and 16 weeks. F2: males pups 1.3 mg/L: As in F1 males, earlier descent of testis was noted: day 16 (42%), day 17 (40%), day 18 (15%) vs. control on day 16 (10%), day 17 (39%), day 18 (31%), day 19 (14%).	Experimental result 2 (reliable with restrictions). Weight of evidence. Test material: Methanol.	Takeda K. and Katho N., 1988
Rats (Sprague-Dawley) Concentrations: 0-10.000-20.000ppm (0-13.100-26.200mg/m3 GD:1-19 at 0-10,000 for 7h/day GD:7-15 at 20.000 for 7h/day 0-5000 ppm (0-6.550 mg m3) GD:1-19 at 0-10,000 for 7h/day	Dams: slight unsteady gait only during the first days of exposure no effects on the body weight and food consumption. Foetal: No resorptions 20.000 ppm dose: In total 93% of litters and 54% of foetuses were affected by: Statistically significant weight decrease (female/male control group: 3.15±0.32/3.34±0.36 vs 2.76*±0.47/2.82*±0.56 g.) Statistically significant increase in the incidence of skeletal malformations (0 in the control group vs 72 %) in cranium, vertebrae and ribs and visceral malformations (0 in the control group vs 15 % (in eye, brain-exencephaly and encephaloceles- and cardiovascular and urinary system). 10,000 ppm dose: Statistically significant weight decrease (female/male control group: 3.15±0.32/3.34±0.36 vs 2.93*±0.26/3.12*±0.30 g.), this effect may be caused by the increased number of foetuses. Increase in the incidence of skeletal malformations (0 in the control group vs 2 %) in cranium, vertebrae and ribs and visceral	Experimental result 2 (reliable with restrictions). Weight of evidence Test material: Methanol	Nelson et al. 1985

	malformations (0 in the control group vs 2 %) in eye, brain-exencephaly and encephaloceles- and cardiovascular and urinary system even if not statistically significant. 5000 ppm dose: No adverse effects In conclusion it was observed that the % of litter with abnormal foetuses for 0, 5.000, 10.000 and 20.000 ppm was 0, 15, 47 and 93*%. Foetal NOEL: 5000 ppm Maternal NOAEL: 10000 ppm (as noted by NPT Expert Panel).		

4.11.2.2 Human information.

Table 1 : Summary table on human information

Method	Results	Remarks	References
Human case report Inhalants overdose of primarly carbonator cleaner containing methanol, toluene and isopropanol	A 32-years old, gravid 7, para5 at 32 weeks gestation required Cesarean section. The course of her current pregnancy had been significant for eight hospital admission for inhalants overdose(primarily carbonator cleaner containing methanol, toluene and isopropanol). A 1570 gr male foetus was delivered via the Cesarean incision and non maternal and neonatal postoperative complications were reported.	Weight of evidence	Kuczkowski K.M. and Le K., 2004
Human case study Ingestion	A 28-year-old woman, gravid 3, para 2, EGA 30 weeks, with HIV infection, asthma, and history of cocaine use and hospitalization, two months earlier for unexplained metabolic acidosis and lethargie and in respiratory distress. Due to the mother's altered mental status the reason and time of her exposure remain unknown. The history of a previous hospitalization with an undiagnosed acidosis might have suggested a repetitive behavior such as methanol ingestion The high anion gap metabolic acidosis in the newborn was likely due to several factors: 1) formic acid from the fetal metabolism of methanol, 2) prolonged maternal acidosis, 3) lactate produced from methanol methabolism and 4) poor tissue perfusion. A formic acid level was not measured on the newborn, therefore no comment on extent of	Weight of evidence	Belson M. and Morgan B.W., (2004)

	the metabolic process has been made		
Human case study Inhalation	A woman exposed repeatedly during pregnancy (16 and 27 weeks of gestation) was admitted to the hospital because of acute intoxication (severe anion gap hyperosmolar metabolic acidosis showing blood methanol levels of about 450 mg/l). At 31 weeks of gestation she was found obtunded and given sodium bicarbonate, to correct acidosis, and ethanol, followed by an emergency Cesarean section for acute foetal distress. At birth, the infant was of appropriate weight but presented acute foetal distress with significant metabolic acidosis. Initial hypotonia was followed by generalized hypertonicity of lower extremities within a week after birth. Neurosonogram showed bifrontal cystic lesions in the frontal area. The frontal cysts measured 1 cm x 1 cm on the right side and 0.8 cm x 0.9 cm on the left side. Magnetic resonant imaging performed on day 3 after birth showed extensive bifrontal cystic leukomalacia with some cortical atrophy and the areas of leukomalacia not communicating with the ventricles. Ventricular size was normal. There was no midline shift. The infant passed an initial hearing screen for both ears.	Supporting sudy	Bharti D., 2003
Human Clinical case study Intentional exposure	Fifty-six patient with a diagnosis of solvent abuse (including MeOH) in pregnancy present to a Manitoba teaching hospital. Twelve patients of 56 mothers with a diagnosis of solvent (including MeOH) abuse in pregnancy showed preterm birth (21.4%), nine infants had major anomalies (16.1%), seven infants had fetal alcohol syndrome-like facial features (12.5%) and six neonates had hearing loss (10.7%). Substance abuse in pregnancy is associated with severe maternal and neonatal sequelae. Physicians must be aware of this increasing problem in the obstetrical population and assistance should be offered to each woman, ideally before a woman becomes pregnant, but at least at the first contact a pregnant woman makes with the health care community.	Weight of evidence	Scheeres J.J. and Chudley A.E., 2002
Human Occupational	Information about the occupational exposure of 851 women (100 mothers of babies with	Supporting study	Lorente et al., 2000

exposure (inhalation and cutaneous)	oral clefts and 751 mothers of healthy referents) who worked during the first trimester of pregnancy was obtained from an interview. This interview was blindly reviewed by industrial hygienists, who assessed the presence of chemicals and the probability of exposure. All women were part of a multicenter European case-referent study conducted using 6 congenital malformation registers between 1989 and 1992. The odds ratio (OR) for cleft lip (with or without cleft palate) was 3.61 (95% CI 0.91-14.4). Due to the limited number of subjects, the committee is of the opinion that this result must be interpreted with caution.		
Human (ingestion; 250-500 ml methanol in the 38th week of pregnancy)	Five hours after methanol ingestion, the woman was slightly acidotic and had a serum methanol level of 2300 mg/l and a formic acid concentration of 336 mg/l. Treatment consisted of ethanol and bicarbonate administration together with hemodialysis. Six days later, the woman gave birth to an infant with no signs of distress. A 10-year follow-up of the child revealed no visual disturbances.	Weight of evidence	Hantson et al., 1997
Human case study Ingestion	Maternal acidosis which occurs following the ingestion of methanol has more serious consequences going forward the pregnancy: this is because the immature foetus is incapable of generate toxic metabolite and maternally produced metabolite (formate) is an unlikely candidate for transplacentar passage. Risk increase with age during the second half of gestation with the maturation of specific metabolizing enzymes. Nonetheless the foetus at any age is at risk when exposed to prolonged maternal acidosis because of resultant of fetal acidosis or severe disruption of maternal homeostasis.	Weight of evidence	Tenenbein M., (1997)

4.11.2.3 Other relevant information: in vitro studies

Table 1: *in vitro* studies

Method	Results	Remarks	Reference
Whole embryo culture:C57BL/6J mouse embryos expressing human catalase (hCat);	CRL (crown-rump lenght): >19% in MeOH exp. hCat compared to NaCl exp. hCat; < 37% in MeOH exp. aCat compared to MeOH exp. C3H WT; no significant variation between MeOH exp. aCat and	Experimental result 4 (not assignable) Supporting study Test material: MeOH	Miller and Wells, (2011)

C57BL/6 wild-tipe mouse embryos (C57 WT); C3Ga.Cg-Catb/J acatalasemic mouse embryos (aCat); C3HeB/FeJ wild-tipe mouse embryos (C3H WT). Dose level0 (NaCl vehicle) and 4 mg/ml of MeOH Exposure: 24 hours. (A single exposure was performed.)	NaCl exp. aCat and between MeOH exp. WTs and NaCl exp. WTs. Anterior neuropore closure: <60% in MeOH exp. C57 WT compared to NaCl exp. C57 WT; no significant variation between MeOH exp. hCat and NaCl exp. hCat; <15% in MeOH exp. C3H WT compared to NaCl exp. C3H WT; <100% in MeOH exp. aCat compared to NaCl exp. aCat and MeOH exp. C3H WT. Turning: <69% in MeOH exp. C57 WT compared to NaCl C57 WT; no sign. variation between MeOH exp hCat and NaCl exp. hCat; <33% in NaCl aCat compared to NaCl C3H WT; <23% in MeOH exp. C3H WT compared to NaCl C3H WT; <27% in MeOH exp. aCat compared to NaCl aCat. Somite development: <13% in MeOH exp. C57 WT compared to NaCl exp. C57 WT; no significant variation between MeOH exp. hCat compared to NaCl exp. hCat; <13% in MeOH exp. C3H WT compared to NaCl exp. C3H WT; <21%in MeOH exp. aCat compared to NaCl exp. aCat.) Yolk sac diameter: No significant variation between MeOH exp. hCat and NaCl exp. hCat and MeOH exp. C57 WT; <15% in NaCl aCat compared to NaCl C3H WT; <13% in MaOH aCat compared to MeOH C3H WT; no significant variation between MeOH exp. and non-exp. WTs. Heart rate:>31% in MeOH exp. C57 WT compared to NaCl exp. C57 WT; >51% in MeOH exp. hCat compared to NaCL exp. hCat; no significant variation between MeOH exp. aCat and NaCl exp. aCat and between MeOH exp. C3H WT and NaCl exp. C3H WT. Head length:<14% in MeOH aCat compared to NaCL aCat; no significant variation between MeOH C3H WT and NaCl C3H WT. Comparison of growth of hCat and C57BL/6 WT saline-exposed embryos: No differences in any parameters were observed for baseline embryonic growth and development between saline-exposed hCat and C57BL/6 WT embryos. MeOH embryopathies in C57BL/6 WT embryos: Exp. to 4 mg/ml MeOH for 24 h resulted in dysmorphogenesis evidenced by significant decreases in anterior neuropore closure (60%), turning (69%) and somite development (13%), along with a significant increase in heart rate (31%), compared to NaCl exp. WT. MeOH embryopathies in hCat embryos: MeOH was embryopathic in hCat embryos, evidenced by significant increases in crown-rump length (19%) and heart rate (51%)		

	compared to saline-exposed hCat controls. Comparison of MeOH embryopathies in hCat vs C5BL/6 WT embryos: Compared to MeOH-exposed WT controls, hCat embryos were almost completely protected from MeOH embryopathies, as evidenced by increases back to saline control levels for anterior neuropore closure ($p<0.05$), somite development ($p<0.05$) and turning ($p=0.1$) Comparison of growth of aCat and C3H WT saline-exposed embryos: There was a significant decrease in yolk-sac diameter (15%) in aCat embryos compared to WT embryos exposed to saline vehicle. Non-significant trends were apparent for decreased turning (33%), and possibly anterior neuropore closure (20%). MeOH embryopathies in C3H WT embryos: Exposure to MeOH for 24 h resulted in dysmorphogenesis evidenced by a significant decrease in somite development (13%), with non-significant decreases in anterior neuropore closure (15%) and turning (23%), compared to saline-exposed WT controls. MeOH embryopathies in aCat embryos: MeOH was highly embryopathic in aCat embryos, evidenced by significant decreases in anterior neuropore closure (100%), somite development (21%) and head length (14%), along with a nonsignificant decrease in turning (27%), compared to saline-exposed aCat. Comparison of MeOH embryopathies in aCat and C3H WT embryos: aCat embryos were more susceptible than WT controls to MeOH embryopathies, evidenced by decreased anterior neuropore closure (100%) ($p<0.05$), yolk-sac diameter (13%) ($p<0.05$) and crown-rump length (37%) ($p=0.05$) in aCat embryos compared to MeOH-exp. WT. Comparison of MeOH embryopathies in C3H WT versus C57BL/6 WT embryos: C3H WT strain was more resistant to MeOH embryopathies than the C57WT strain, the latter of which exhibited a greater extent and severity of embryopathies. In conclusion all these data suggest that ROS may be involved in the embryophatic mechanism of MeOH, and that embryonic catalase activity may be a determinant of teratological risk.		
Ex vivo study on embryo mouse and rat Exposure microinjection Mouse/CD-1/GD8 at 4 - 12 mg/mL for 24 hrs Rat/Sprague-	Mouse: Reduced VYS DNA and rotation at 4 mg/mL; reduced embryo DNA and protein, neural tube closure and viability at 8 mg/L; reduced VYS protein at 10 mg/L. Rat: Reduced embryo protein and rotation at 8 mg/mL; reduced VYS DNA and protein,	Experimental result 2 (reliable with restrictions) Weight of evidence Test material: MeOH	Hansen et al., (2005)

Dawley/GD10 at 8 - 20 mg/mL for 24 hrs	embryo DNA, and neural tube closure at 8 mg/L; reduced viability at 16 mg/L.		
Mouse (CD-1 and C57BL/6J) 0, 1, 2, 3, 4, 6 mg/ml of MeOH inserted in culture media. Exposure: the conceptuses were placed in culture media containing MeOH for 24 hours. A single administration at different concentration of MeOH was used.	On C57BL/6J embryos. -At 4 mg MeOH/ml exposure: embryos had total protein, incomplete rotation, reduced prosencephalon, cranial neural tube open and eye dysmorphology which were significantly lower than those found in controls. -At 6 mg MeOH/ml exposure: embryos had somites, total protein, incomplete rotation, reduced prosencephalon, cranial neural tube open, eye dysmorphology and cranial neural tube open. On CD-1 embryos: -at 6 mg MeOH/ml exposure: embryos had somites, total protein, incomplete rotation, reduced prosencephalon, cranial neural tube open and eye dysmorphology as the C57BL/6J embryos at 4 mg MeOH/ml. -At 4 mg MeOH/ml exposure: embryos had reduced prosencephalon and eye and heart dysmorphology. Lysotracker red staining showed cell death in embryos cultured for 8 hours on C57BL/6J embryo: -at 4 mg MeOH/ml exposure embryos showed an increased intensity of staining in the dorsal hindbrain. -at 6 mg MeOH/ml exposure embryos showed intense areas of staining in the neural folds. Lysotracker red staining showed cell death in embryos cultured for 8 hours on CD-1 embryo: -at 6 mg MeOH/ml exposure, embryo exposed showed staining in the craniofacial region, but less than in the C57BL/6J embryo exposed to the same concentration of test material. -at 4 mg MeOH/ml exposure, embryos showed staining in the forebrain, hindbrain, eye and otic pit. Lysotracker red staining showed cell death in embryos cultered for 18 hours on C57BL/6J embryo: -at 6 mg MeOH/ml exposure, embryos showed an intense staining in the forebrain, eye, hindbrain and optic pit and an increase in staining in the trigeminal ganglia. Lysotracker red staining showed cell death in embryos cultured for 18 hours on CD-1 embryo: -at 6 mg MeOH/ml exposure, cell death in the forebrain and hindbrain, and in the region of the trigeminal ganglion. Cell death plays a prominent role in MeOH induced dysmorphogenesis, while cell-cycle perturbation may not. Differences in the	Experimental result 4 (not assignable) Supporting study Test material: MeOH	Degitz et al., (2004)

	extent of cell death between CD-1 and C57BL/6J embryos correlated with differences in the severity of dysmorphogenesis.		
Rat (Sprague-Dawley) whole embryo culture MeOH: 12 and 24 mg/ml; formaldehyde: 3 and 5 µg/ml; sodium formate: 0.5 and 2 mg/ml; BSO (as inibitory of GSH synthesis): 2 mg/ml Exposure: 24 h Whole embryo culture studies were conducted using GD 10-11 rat.	-At 12 mg/ml based of MeOH exposure: significantive alteration in viability, neuropore closure, crown-rump length, number of somites and embryonic bloody blisters were observed. -At 24 mg/ml based of MeOH exposure: significative alteration in viability, neuropore closure, crown-rump length, number of somites and embryo appeared necrotic and with bloody blisters were observed. -At 2 mg/ml based of BSO exposure: significative alteration in crown-rump length was observed. -At methanol (12 mg/ml) + BSO (2 mg/ml) exposure: significant in comparison with MeOH alone treatment group - alteration in rotation, crown-rump length and number of somites were observed. -At MeOH (24 mg/ml) + BSO (2mg/ml) exposure: significant in comparison with MeOH alone treatment group - alteration in rotation, neuropore closure, crown-rump length, and embryonic bloody blisters were observed -At 3 µg/ml based of formaldehyde exposure: significative alteration in viability and rotation was observed. -At 6 µg/ml of formaldehyde exposure: significative alteration in viability, rotation, neuropore closure, crown-rump length and embryonic bloody blisters were observed. -At 2 mg/ml based of BSO exposure: significative alteration in crown-rump length was observed. -At 3 µg/ml based of formaldehyde (3 µg/ml) + BSO (2mg/ml) exposure: significant in comparison with formaldehyde alone treatment group - alteration in viability, rotation, neuropore closure, number of somites and embryo appeared necrotic and with bloody blisters were observed. -At 6 µg/ml based of formaldehyde (6 µg/ml) + BSO (2mg/ml) exposure: all embryos were deaths. -At 0.5 mg/ml of sodium formate exposure: significative alteration in viability and bloody blisters were observed. -At 2 mg/ml of sodium formate exposure: significative alteration in viability, number of somites and embryo appeared necrotic were observed. -At 2 mg/ml of BSO exposure: significative alteration in crown-rump length and embryonic bloody blisters were observed.	Experimental result 4 (not assignable) Supporting study Test material: MeOH (moreover, formaldehyde, sodium formate and L-buthionine-S,R-sulfoximine (BSO) were used in the study)	Harris et al., (2004)

	-At 0.5 mg/ml based of sodium formate (0.5 mg/ml)+ BSO (2mg/ml) exposure: significant in comparison with sodium formate alone treatment group - alteration the number of somites and embryo appeared necrotic and with bloody blisters. -At 2 mg/ml of sodium formate (2 mg/ml) + BSO (2mg/ml) exposure: significant in comparison with sodium formate alone treatment group - alteration in viability and embryo appeared necrotic. The data showed that MeOH is dismorphogenic and that gluthatione is important in the detoxication of MeOH in the developing foetus.		
Rat and mouse (Sprague-Dawley and CD-1) The MeOH, ethanol and formaldehyde are inserted in the samples for the enzyme assays. 50 µl of MeOH added to tissue omogenate. 9 µl of Ethanol added to tissue omogenate. 10 µl of Formaldehyde added to tissue omogenate. Exposure: Embryos were not exposed to MeOH; the substances were added to embryos tissues to assess the activity of the enzymes of interest.	Variation of Catalase-specific activities (embryos, VYSs, heads, hearts, trunks): -at 50 µl of MeOH: Catalase-specific activities increased as organogenesis proceeded in both rat and mouse conceptuses. Catalase-specific activity in rat heart was found to be greater than two-fold higher than in mouse heart at the 6–12-somite stage. -at 9 µl of Ethanol: ADH1 activities were significantly lower by 25% in the mouse embryo at the early stage. VYS ADH1 activity in both the mouse and rat showed very similar developmental activity but rat VYS ADH1 activities were 15–25% higher than those seen in the mouse. -at 10 µl of Formaldehyde: Comparisons between species indicate that the rat VYS contained significantly increased ADH3 activity. Comparison of embryonic tissues showed that only heart ADH3 activity was different between species in young embryos. Other tissues were not different.	Experimental result 4 (not assignable) Supporting study Test material: MeOH (moreover ethanol, formaldehyde were used in the study)	Harris et al., (2003)
Mouse (CD-1) whole embryo culture 0, 4, 8 mg/ml of MeOH Exposure: 24 h in culture medium.	Increasing in DNA methylation at 0, 4, 8 mg MeOH/ml exposure. The embryonic DNA had 30% (control group), 54% (4 mg/ml) and 30% (8 mg/ml) of methylation. Inhibition of specific protein synthesis at 4 mg/ml and 20 µCi/ml ^{14}C-MeOH. ^{14}C-MeOH exposure: no inhibition of specific protein synthesis was apparent at this concentration of MeOH. Protein fractions analyzed gave similar profile in control and treated group for both embryos and yolk sacs. Radiolabeling of DNA: 0 — 8 mg MeOH/ml exposure. There was significant radiolabeling of DNA following embryonic exposure for 24 h to ^{14}C-MeOH; the embryonic DNA peak was correlated with the ^{14}C activity demonstrating that ^{14}CMeOH was incorporated into DNA (under experimental conditions). Changing in protein profile: based on: ^{14}C-MeOH in presence of 35S-Methionine:	Experimental result 4 (not assignable) Supporting study Test material: MeOH	Huang et al., (2001)

	Comparison of the radiolabeled protein profiles obtained from 35S-methionine exposure and ^{14}C-MeOH exposure indicated that all newly synthetized proteins were labelled by both radiolabels. These results indicate that methyl groups from ^{14}C-MeOH are incorporated into mouse embryo DNA and protein. These results further suggest that MeOH exposure may increase genomic methylation under certain conditions which could lead to altered gene expression.		
Ex vivo Study Virgin Sprague-Dawley rats (Crl:CD [SD] BR) (GD 9) rat embryos were exposed to various concentrations of MeOH and formate in whole embryo culture (WEC) for 48 hr and the degree of embryotoxicity was evaluated using developmental score (DEVSC) as the parameter of comparison across exposure combinations. The concentrations of MeOH and formate used separately and in combination ranged from 0 to 8.75 mg/ml MeOH and 0 to 1.51 mg/ml formate.	The concentrations of MeOH and formate chosen for simplex 1 were calculated to give a DEVSC value which was approximately 86.5% of the control value, whereas the concentrations chosen for simplex 2 were calculated to give a DEVSC value which was approximately 73% of the control value. The two groups of embryos grown in mixtures had DEVSC values that were significantly higher than those for the embryos exposed to formate or MeOH alone. Low concentrations of formate (up to 1.00 mg/ml), along with various concentrations of MeOH, did not result in a significant decrease in the DEVSC below that which would be expected from exposure to that concentration of MeOH alone. Higher concentrations of formate (.1.00 mg/ml), in combination with the indicated concentrations of MeOH, resulted in significant reductions of embryonic DEVSC.	Experimental result 4 (not assignable) Supporting study Test material MeOH	Andrews et al., (1998)
Rat and mouse (Sprague-Dawley and CD-1) whole embryo culture Dose levels rat: 0, 8, 12, 16 mg/ml - mouse: 0, 2, 4, 8 mg/ml Exposure: Rats: 24 and 48 h. Mice: 24 h.	Abnormalities in rat embryos in growth and developmental parameters: -at 0 (control group) and 8 mg/ml exposure 24/24 h: - no significative alteration in all parameter were observed. -at 12 mg/ml exposure 24/24h: significative alteration in yolk sac diameter and number of somites were observed -at 0 (control group) exposure 48/48h and at 8 mg/ml exposure 24/48h: no significative alteration in all parameter were observed. -at 12 mg/of exposure 24/48h: significative alteration in number of somites were observed. -at 16 mg/ml exposure 24/48h: significative alteration in head length and developmental score were observed. -at 8 mg/ml exposure 48/48h: significative alteration in developmental score was observed.	Experimental result 4 (not assignable) Supporting study Test material: MeOH	Abbott et al., (1995)

	-at 12 mg/ml exposure 48/48h: significative alteration in yolk sac diameter, head length, developmental score and number of somites were observed. Abnormalities in mouse embryos in growth and developmental parameters: -at 0, 2 and 4 mg/ml exposure 24h: no significative alteration in all parameter were observed. -at 8 mg/ml exposure 24h: a significative alteration in crown rump length, head length, developmental score and number of somites were observed. Rat whole embryo culture: incidence of cell deaths in specific region: -at 0, 8 and 12 mg/ml exposure 24/24h; at 0, 8 and 16 mg/ml exposure 24/48h : no significative cell deaths in all region were observed. -at 12 mg/ml exposure 24/48h: significative cell deaths in optic placode were observed. -at 12 mg/ml exposure 48/48 h: significative cell deaths in Visceral arch No. 2, Otic placode were observed. -at 16 mg/ml exposure 48/48h: significative cell deaths in forebrain, optic placode, visceral arch no. 1, visceral arch no. 2, optic placode (all region) were observed.		
Rat (Sprague-Dawley). Embryo culture MeOH, toluene, formic acid, sodium formate and hydrochloric acid were inserted in culture media 0-450.0 µmol/ml of MeOH 0-3 µmol/ml of toluene 0-30.0 µmol/ml formic acid 0-20 µmol/ml sodium formiate HCl concentrations were chosen to achieve the pH either similar to or lower than that achieved by addition of formic acid. Exposure: Cultures for each solvent concentration were done over at least 2 separate days and for each day, embryos from at least 3 litters were pooled and the embryos randomly assigned to the culture bottles.	-At 286.5 ± 1.7µmol /ml (9.18±0.05 mg/ml) of MeOH exposure: reduced the n. of embryos with well-developed yolk sac blood vessels (44.4%), decreased crown-rump lenght, somite number and total protein was observed. -at 411.7± 49.9 µmol /ml (13.19±1.60 mg/ml) of MeOH exposure: reduced the n. of embryos with well-developed yolk sac blood vessels (0%), fully dorsally convex (70%), decreased crown-rump lenght, somite number and total protein was observed. -At 346.8 µmol /ml (11.11 mg/ml) of MeOH exposure the n. of embryos with well-developed yolk sac blood vessels (20%) was reduced; decreased crown-rump lenght, somite number and total protein was observed. -At 18.66 µmol/ml (0.86 mg/ml) of formic acid exposure. The embryos showed a decrease in crown-rump lenght, somite number , total protein and ending pH . -At 27.96 µmol /ml (1.29 mg/ml) of formic acid exposure. Embryos showed a decrease in crown-rump lenght, somite number, total protein and ending pH. -At 18.7 µmol /ml (1.27 mg/ml) of sodium formate exposure, Embryos showed a decrease in crown-rump lenght , somite numberand total protein. Ending pH was 7.44±0.07, higher than the control.	Experimental result 4 (not assignable) Supporting study Test material: MeOH (moreover toluene, formic acid, sodium formate, hydrochloric acid were used in the study).	Brown-Woodman et al., (1995)

A single administration at different concentration of each test material was used.	-At 18.7 µmol/ml of sodium formate 1.27 mg/ml + formic acid 1.07 mg/ml , Embryos showed a decrease in crown-rump lenght, somite number and total protein.Ending pH was 7.46±0.12, higher than control.observed. All the data showed that both MeOH and formic acid have a concentration-dependent embryotoxic effect on the developing rat embryo in vitro.		
Mouse (CD-1) cultures. 6,8,10,12,15,18,20 mg/ml of MeOH; 3,3.5,4,5,10,15 mg/ml of Ethanol; Exposure: MeOH exposure lasted either 6 hours, 12 hours, 1 day or 4 day. Ethanol exposure lasted 4 day.	Statistically significant effects of abnormal fusion and morphology: The palates exposed to 20 mg/ml of MeOH for 1 day which did not fuse (57%) had extensive epithelial degeneration along the entire medial edge which left the underlying mesenchyme exposed. Effects on Proliferation and Growth (Level of PROTEIN): >= 6 - <= 20 mg/ml of MeOH: A significant dose-related decrease in total protein was detected with exposures lasting 12 hours or longer. No change was detected after only 6 hours of MeOH at any concentration tested. The effects on protein were more severe after 1 and 4 days. A significant dose-related decrease in total DNA occurred after MeOH exposure lasting for 6 hours or longer. After 6, 12 hours, and 4 days of MeOH treatment, the effects on total DNA level were significantly greater than effects on protein. Exposure to 12 hours showed a trend for significant increase in 3H-TdR uptake; tissues exposed continuously for 4days had significantly decreased uptake. The protein/DNA ratio significantly increased relative to controls for the 6, 12 hour, and 4 day groups, but was decreased with 1 day of exposure. The increase seen for 6, 12 hours, and 4 days did not differ significantly between these groups.	Experimental result 4 (not assignable) Supporting study Test material: MeOH (ethanol was used in comparison with methanol)	Abbott et al., (1994)
CD-1 mouse and Sprague-Dawley rats whole embryo culture 0, 2, 4, 8, 12, 16 mg MeOH/ml serum in Rat 0, 2, 4, 6, 8 mg MeOH/ml serum in.Mouse Exposure: 24h	Abnormal embryos in rats: -At 12 mg/ml a significant increase was observed (66%). No significant effect were observed at lower tested doses (0-8 mg/ml). Abnormal embryos in mice: -at 6-8 mg/ml, a significant increase was observed, 58% at 6 mg/ml and 80% at 8 mg/ml. No significant effect were observed at lower tested doses (0-4 mg/ml). Embryolethality: -At 12 -16 mg/ml a significant increase was observed in rats (at 12 mg/ml was 53% and 95% at 16 mg/ml). -at 6 - 8 mg/ml, a significant increase was	Experimental result 4 (not assignable) Supporting study Test material MeOH	Andrews et al., (1993)

	observed in mice (31% at 6 mg/ml and 89% at 8 mg/ml). Developmental score in rats: -at 8 mg/ml a significant decreases was observed; -at lower tested doses 0-4 mg/ml no significant effects were observed; Developmental score in mice: -At 2 mg/ml a significantly lower developmental score than controls was observed. Crown-rump length in rats: -at 8 mg/ml a significant decrease was observed; -at 0-4 mg/ml, no significant effects were observed. Crown-rump length in mice: -at 2 mg/ml a significantly lower crown-rump length was observed.Yolk sac diameter in rats: -at 8 mg/ml a significant decrease was observed; -at 0-4 mg/ml, no significant effects in yolk sac diameter were observed. Yolk sac diameter in mice: -At 4 mg/ml a significant decrease was observed.-At 0-2 mg/ml tested doses, no significant effects were observed. Somite number in rats: -at 8 mg/ml a significant decrease observed.-at 0-4 mg /ml, no significant effects were observed; Somite number in mice: -at 4 mg/ml a significantly decreased was observed -at 0-2 mg/ml, no significant effects were observed. Head length in rats: -at 8 mg/ml a significant decrease was observed -at 0-4 mg/ml, no significant effects were observed. Head lengthin mice: -at 4 mg /ml a significant decrease was observed. -at 0-2 mg /ml tested dose, no significant effects were observed. Embryonic protein contentin rats: -at 0-12 mg /ml protein content of the embryos rats was not significantly affected. Embryonic protein contentin mice: -at 6 mg /ml significantly decreased. -At 0-4 mg/ml no significant effects were observed. Effects observed in the surviving embryos of the higher dose group in rats: -at 16 mg /ml, the effects observed in the surviving embryos were delayed limb, bud development, abnormal brain development and open neural tube. Anomalies observed in the controls:		

	-at 0 mg /ml and at lower MeOH levels the effects observed were delayed development, effects on rotation, limb bud development and erratic neural seam. A total of eight lobules from different placentae were perfused with formic acid (four with folate added and four without folate added) and the physical parameters for the perfusions are given. Formic acid transferred rapidly from the maternal to the fetal circulation. In the presence or absence of folate to the perfusate, formic acid appeared in the fetal circulation within 10 min in all eight perfusions. The addition of folate into the perfusate did not alter the fetal AUC (1.30 ± 0.14 without folate; 1.23 ± 0.48 with folate; P = 0.79). Tissue concentrations of formic acid measured in the perfused lobules at the completion of the experiment were 425.83 ± 57.18 and 431.18 ± 133.07 nmol/g for perfusions without and with folate added, respectively. Compared with the pre-experimental control period, there was a significant decrease in the rate of hCG secretion in the maternal circulation after the addition of formic acid in the experimental period (P = 0.03). The percentage of initial placental tissue hCG was decreased in the perfusions without folate compared with perfusions with folate (P = 0.04) The addition of folate did not alter the transfer of formic acid; however, it did mitigate the effects on hCG secretion. Since tissue concentrations of formic acid were similar in the presence or absence of folate, this suggests that folate may mitigate toxicity to the placenta by acting as an antioxidant to the oxidative stress caused from formic acid as opposed to increasing clearance of formic acid. Conclusions: Formic acid rapidly transfers across the placenta and thus has the potential to be toxic to the developing foetus. Formic acid decreases hCG secretion in the placenta, which may alter steroidogenesis and differentiation of the cytotrophoblasts, and this adverse effect can be mitigated by folate.		

4.12 Summary and discussion of reproductive toxicity

4.12.1 Effects on development

Animals

Pre-natal developmental toxicity of MeOH was studied in rats, mice and rabbits after inhalatory or oral (gavage or drinking water) exposure.
In general, in rodents pre-natal developmental toxicity was evidenced by decreased foetal weight, decreased incidence of live foetuses and increased incidences of resorptions and dead foetuses, as well as by teratogenic effects: neural tube defects, cleft palate, skeletal (cranium, vertebrae, ribs, limb, tail) and visceral (eye, brain, cardiovascular and urinary system) malformations (Nelson et al., 1985; Dorman et al. 1995; Rogers and Mole, 1997; De-Carvalho et al., 1994; Connelly and Rogers, 1997; Sweeting et al., 2011).
In a number of studies (Nelson et al., 1985; Bolon et al. 1993; De-Carvalho et al., 1994; Fu et al., 1996; Connelly and Rogers, 1997) developmental toxicity was observed without overt signs of maternal toxicity. At higher concentrations, more severe developmental effects were observed in combination with maternally toxic effects: decreased body weight or weight gain, neurological symptoms (unsteady gait, ataxia, circling, tilted heads, depressed motor activity,) (Nelson et al., 1985; Bolon et al. 1993; Dorman et al. 1995; Rogers and Mole, 1997; Sakanashi et al., 1996; Youssef et al., 1997 ; Takeda K. and Katoh N. 1988).
In a two generation study (Takeda K. and Katoh N. 1988) developmental toxicity was observed in F1 and F2 generations, in particular male pups of the 1.3 mg/L group, showed earlier descent testis. Absolute and relative brain weights were significantly lowered of either sex at an age of 8 and 16 weeks. This was still found in females necropsied after 24 weeks.
In the study with rabbit (Sweeting et al., 2011), although was a non-standard experiment (2 mg/Kg bw, 2 times/day, on gestational day 7 or 8, i.p. administration), the results showed an increase of malformations, mainly tail abnormalities, without overt signs of maternal toxicity. Therefore, the study suggests that MeOH may act as teratogen also in non-rodents.
Post-natal developmental toxicity of MeOH was studied in rats (Stanton et al.,1995; Stern et al., 1997; Infurna and Weiss, 1986) and in Macaca fascicularis monkeys (Burbacher et al., 1999; Burbacher et al., 2004). In the offspring of both rats and monkeys, some effects were observed on neurobehavioural parameters, but the evidence is not robust enough to indicate MeOH as a toxicant impairing neurobehavioral development.
In the whole-pregnancy study on non human primates (Macaca fascicularis), maternal exposure to inhaled MeOH (200, 600, or 1800 ppm, 2.5 h/day, 7 days/week prior to breeding and throughout pregnancy) induced a consistent reduction in length of pregnancy (6-8 days) accompanied by a presence of pregnancy complications (bleeding at parturition, stillbirths) without overt signs of maternal toxicit: the changes were present at all exposure levels without significant differences among levels, thus a NOAEC was not identified. The authors hypothesize a MeOH-induced perturbation of fetal hypothalamus-pituitary-adrenal axis regulating late pregnancy and delivery in mmany mammalian species including primates (Burbacher et al., 2004).

In vitro and mechanistic studies

The in vitro developmental toxicity studies performed by using rat and mouse whole embryo culture assays, confirmed the MeOH induced abnormal morphogenesis observed in vivo in rodents (Degitz et al., 2004; Harris et al, 2004; Harris et al., 2003; Huang et al., 2001; Brown-Woodman et al.,1995; Abbott et al., 1994; Abbott et al., 1995; Andrews et al., 1993). A complex in vitro study (Miller and Wells, 2011) comparing mouse embryos of different strain, including mice expressing human catalase or not expressing catalase at all, showed that:

i) Reactive Oxygen Species (ROS) production is important in the MeOH-induced dysmorphogenesis;
ii) the activity of mouse embryonic catalase is inversely related to MeOH dysmorphogenic effect;
iii) mouse embryos expressing human catalase were protected from dysmorphogenic effects, although they showed some significant effects on growth.

Further to ROS production, reduced uteroplacental blood flow leading to conceptus hypoxia may contribute to the MeOH prenatal toxicity, as observed by Ward and Pollack (1996) in a study on rats and mice at mid- or term pregnancy using intrauterine microdyalisis (Ward and Pollack , 1996).

Formic acid as toxic metabolite of MeOH

Formic acid is a toxic metabolite of MeOH in mammals, leading to acidosis. Formic acid accumulation occurs in human, rabbit and primates but not in rodents. Formic acid metabolism occurs through a folate-dependent pathway; primates have lower folate concentrations than rodents, thus may accumulate formic acid, whereas rodents can metabolize formic acid through CAT and excrete it as water and CO_2. Formic acid has been reported in maternal blood and umbilical cord blood of infants born from heavy drinkers (Hutson et al., 2013). *In vitro* studies on rat and mouse showed that formic acid can cause a spectrum of embryotoxic effects (growth restriction, lethality, to a lesser extent dysmorphogenesis) comparable to MeOH (Brown-Woodman et al., 1995; Andrews et al., 1998; Harris et al., 2004; Hansen et al., 2005); both MeOH and formic acid cause a significant depletion of the antioxidant glutathione in both cultured rat embryos and yolk sac, lending further support to the role of ROS in MeOH embryotoxicity (Harris et al., 2004). A recent study investigated the placental transfer and effect of FA in human placental explants *ex vivo*. Formic acid is transferred rapidly from the maternal to the fetal circulation, and transfer was not altered with the addition of folic acid. Formic acid also elicited a significant decrease in human chorionic gonadotropin (hCG) secretion, that was mitigated by the addition of folic acid (Hutson et al., 2013).

Overall, the *in vivo* and *in vitro* investigations suggest that rodents may be more susceptible to MeOH developmental toxicity than non-rodent species, including humans; however, MeOH developmental effects may not be unique to rodents.

Humans

Limited data are available concerning the effects of exposure to MeOH on development in humans; most of them concern case reports upon intoxication of pregnant women. (Hantson et al, 1997; Bharti, 2003; Belson and Morgan, 2004; Tenenbein, 1997; Kuczkowski and Le, 2004). For instance, a woman intoxicated at 38th week of gestation with MeOH gave birth to an infant with no signs of distress six days after intoxication (Hantson et al.,1997). This is not surprising since the pregnancy to term was actually completed. Another woman gave birth to an infant presenting acute foetal distress with significant metabolic acidosis and cerebral infarcts after exposure to a mixture of solvents containing MeOH (Bharti, 2003).

Lorente et al. (2000) found inconclusive results on the incidence oral clefts after occupational exposure to MeOH during the first trimester of pregnancy. The newborns from 56 mothers with a diagnosis of solvent (including MeOH) abuse in pregnancy showed preterm birth (21.4%), major anomalies (16.1%), fetal alcohol syndrome-like facial features (12.5%) and hearing loss (10.7%) (Scheeres and Chudley, 2002).

Overall, it is to be noted that the findings are inconclusive concerning the developmental toxicity of MeOH in humans due to too much confounding factors.

4.13 Summary of MeOH development effects

The proposal for classification is based on the added value of weight of evidence, as provided by the integrated assessment of the available studies. Therefore all studies considered contribute to the proposed classification.

Based on animal studies, severe developmental effects are consistently recorded in both rats and mice in absence of maternal toxicity.

In general, prenatal developmental toxicity was evidenced by decreased foetal weight, decreased incidence of live foetuses and increased incidences of resorptions and dead foetuses, as well as teratogenic effects (neural tube defects, cleft palate and skeletal and visceral malformations). Moreover, post-natal effects (also observed at maternally toxic dose levels) included increased neonatal mortality and growth retardation and earlier testis descent; noticeably, exposure to MeOH concurrently increased gestation length.

A recent, non-standard study on the rabbit suggests that MeOH may act as teratogen also in non-rodent species. Therefore, the study does not contradict the MeOH developmental toxicity recorded in species with different MeOH metabolism (such as rodents), albeit the potency might be greater in rodents.

Moreover, in Macaca fascicularis methanol significantly reduced the duration of pregnancy, suggesting that pregnancy represents a susceptible life stage to methanol exposure also in primates.

The mechanisms underlying the developmental effects of MeOH in rodents and in rabbit involve many (and in some cases, alternative) mode of action, although the developmental effects are evident in different species, and may even involve ROS. Mechanistic studies *in vitro* suggest that the activity of mouse catalase is critical for MeOH developmental toxicity, whereas MeOH effects are mitigated, in mouse embryos expressing human catalase *in vitro,* but not abolished. Moreover, it

may be worth noting that the rabbit embryo may be more sensitive than the rat embryo to reactive oxygen-generating toxicants (Hansen et al., 2001), further supporting that ROS-mediated developmental toxicity is not a mode of action unique to rodents. FA, a toxic metabolite of MeOH, produces a comparable spectrum of embryotoxic effects. Since FA metabolism occurs through a folate-dependent pathway, the accumulation of FA is higher in primates than in rodents, due to their lower folate stores.

Placental effects (reduced blood flow, impaired hCG production) may contribute to prenatal toxicity of MeOH (and FA), as shown in rats and mice *in vivo* and in human placental explants *ex vivo*. Noticeably, exposure to MeOH may increase gestation length in rodents.

Overall, *in vivo* and *in vitro* experimental studies suggest that rodents may be more susceptible to MeOH developmental toxicity than non-rodent species, including humans; however, the available evidence supports that MeOH developmental effects are not unique to rodents. The limited human evidence, mainly confined to case reports, can only suggest that high exposure to MeOH during pregnancy may lead to serious foetal and neonatal toxicity: however, no final conclusions can be taken.

4.14 Comparison with criteria

The CLP criteria for classification in Repr.1B are as follow:

"Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)."

Effect on development
Based on animal studies, development is severely impacted in several species (rats, mice, rabbits and monkeys).
In general, prenatal developmental toxicity was evidenced by decreased foetal weight, decreased incidence of live foetuses and increased incidences of resorptions, dead foetuses, exencephaly, neural tube defects, cleft palate and skeletal and visceral malformations. The observation of post-natal adverse effects on neonatal viability, growth and development (earlier testis descent) lend further support to the MeOH as being hazardous for development. Moreover, the available human data on methanol poisoning during pregnancy are limited and inadequate and cannot lead to any conclusion.

A classification Repr.1B – H360D is proposed in the CLP regulation (Repr Cat 2 – R61 for development according to directive 67/548/EEC).

4.15 Conclusions for classifications of MeOH

Taking into account:

i) the clear evidence of developmental toxicity, including teratogenecity, in two species, the rat and the mouse;
ii) that the ability to metabolize MeOH may vary among individuals as a result of genetic, age, and environmental factors;
iii) the supportive evidence on MeOH and FA effects and metabolism in the rabbit and humans;

and based on the weight of evidence and expert judgment, a classification Repr.1B – H360D is proposed in the CLP regulation (Repr Cat 2 – R61 for development according to directive 67/548/EEC).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The proposal for classification was based on weight of evidence from all of the available studies. Severe developmental effects were consistently recorded in both rats and mice in the absence of maternal toxicity. In general, prenatal developmental toxicity was evidenced in these species by decreased foetal weight, decreased incidence of live foetuses and increased incidences of resorptions and dead foetuses (relative to concurrent controls), as well as teratogenic effects (neural tube defects, cleft palate and skeletal and visceral malformations). Moreover, post-natal effects (some of which were observed at maternally toxic dose levels) included increased neonatal mortality and growth retardation and earlier testis descent. A recent, non-GLP, test guideline compliant study in rabbits (Sweeting *et al*., 2011) suggested that methanol may also act as a teratogen in non-rodent species with a metabolic pathway for methanol more similar to humans, albeit the potency might be lower than in rodents. Moreover, in *Macaca fascicularis*, methanol significantly reduced the duration of pregnancy, suggesting that pregnancy also represents a life stage susceptible to methanol exposure in primates. Classification as Repr. 1B – H360D was therefore proposed.

Comments received during public consultation

Three Member State Competent Authorities (MSCAs) supported the proposal. A fourth MSCA opposed the proposed classification based on the large differences in the metabolic pathways of methanol in rodents and humans, and instead suggested classification as Repr. 2, pending a more detailed description of the studies and a more substantial justification.

Three individuals commented on the proposal, with one supporting and two opposing the proposal.

Six industry organisations opposed the proposal. There were two main reasons for the objections. The first concerned kinetic differences (metabolic pathways) between species, making rodents very poor models for methanol toxicity in humans. Accordingly, the CLP guidance uses methanol as an example of when rat data should not be used for classification purposes (concerning acute toxicity and STOT SE). The second reason concerned the very high acute and specific toxicity of methanol in humans, resulting in severe toxicity before reaching such blood concentrations of methanol that led to developmental toxicity in rodents. Based on these arguments, methanol should not be classified for developmental toxicity at all according to the industry organisations.

Additional key elements

Methanol is mentioned as a specific example twice in the current CLP guidance (November 2013; paragraphs 3.1.6.1.1 and 3.8.6.1.1), with the following rationale "*The rat is known to be insensitive to the toxicity of methanol and is thus not considered to be a good model for human effects (different effect/mode of action)*". The first example concerns acute toxicity, for which the lethal dose in humans is 300-1000 mg/kg whereas the LD_{50} in rats is >5000 mg/kg, and the conclusion is that the rat data should be ignored. The second example concerns STOT SE, for which human evidence of blindness caused by relatively low doses of methanol warrants classification, while it is noted that there are no effects on the eyes of rats, even at high exposure levels.

Although not discussed in the guidance, it is likely that the reason for the species differences in toxicity is the different metabolic pathways for methanol in rodents and humans.

Assessment and comparison with the classification criteria

<u>Kinetics/metabolic pathways</u>

The kinetic differences between rodents and humans can be explained by their different sets of enzymes for metabolising methanol, leading to different metabolic rates and different metabolites in rodents and humans. Briefly, the first step in the metabolism of methanol is mediated by catalase and alcohol dehydrogenase in rodents and by alcohol dehydrogenase in primates. The rate-limiting step in rodents is the formation of formic acid, whereas in primates it is the further degradation of formic acid. This results in methanol accumulating in the blood of rodents, while formic acid and methanol accumulate in human blood.

In the human population, it is known that polymorphism in alcohol dehydrogenases exist, leading to differences in sensitivity to Methanol both at the individual level and also at the

Toxicity – human data

There was only one human case reported in the CLH dossier with exposure to "methanol only" (single exposure to 250-500 ml) during late pregnancy, with no effects on the child.

There is, however, more general experience of the acute effects of methanol in humans. The formic acid formed in humans may lead to acidosis, explaining the possibly 10-fold higher acute toxicity of methanol in humans than in rodents. In addition, eye toxicity (potentially leading to blindness) is a very characteristic effect in humans, occurring even at low exposure levels. According to IPCS (2001), acute ingestion of as little as 4 to 10 mL of methanol may cause permanent blindness, but individual susceptibility varies widely, possibly because of the frequent concurrent ingestion of ethanol.

Toxicity – animal data

There are a large number of studies in rats and mice clearly showing developmental toxicity after both oral and inhalation exposure to methanol. I It appears form the dossier that methanol exposure may cause decreased foetal weight, decreased incidence of live foetuses, increased incidences of resorptions, dead foetuses, exencephaly, neural tube defects, cleft palate and skeletal and visceral malformations. However, according to the CLH dossier, the lowest LOAELs/LOAECs were in the order of 1000 mg/kg (1.3 mL/kg) and 5000 ppm, respectively. It is noted that other evaluations have used a mouse developmental toxicity study giving a LOAEC of 2000 ppm as the critical study (inhalation exposure during gestation day (GD) 6-15) (Rogers *et al.*, 1993). The rodent studies showed developmental toxicity, but with a low potency as indicated by the high LOAELs/LOAECs, and the question remains how relevant the rodent data are for humans in the light of the differences in kinetics.

There were also studies in two non-rodent species, which have metabolic pathways more or less similar to the human metabolism of methanol (Sweeting *et al.,* 2010), and which might be important for the assessment of human relevance of the developmental toxicity noted in rodents.

Sweeting *et al.* (2011) dosed rabbits intra-peritoneally with two doses of 2000 mg/kg methanol on GD 7 or 8, and sacrificed the dams at GD 29. The dossier refers to a 4-fold increase in tail abnormalities (short or absent) as the only finding, but RAC notes that the observation was not statistically significant and the poorly reported study is therefore of questionable relevance. The potential methanol-induced developmental toxicity during other parts of the rabbit gestation (than GD 7-8) has not been studied.

Burbacher *et al.* (2004) studied the effects of methanol inhalation (0, 200, 600, 1800 ppm for 2.5 hours daily) on monkeys (*Macaca fascicularis*) for 180 days prior to and throughout their pregnancy. A full study report was published in 1999 by the Health Effects Institute (Burbacher *et al.,* 1999), and the study was later also published in the scientific literature (Burbacher *et al.,* 2004). The four findings included pregnancy complications, shortened pregnancy period, developmental neurotoxicity, and a wasting syndrome. The CLH report contained very limited information, simply concluding that "*methanol exposure was associated with a delay in early sensorimotor development for male infants of all dose groups and with deficits in visual recognition memory for all infants of all dose groups*". Based on this minimal reporting, it was not possible to judge if there is a dose-response relationship (incidence, severity) and thus whether these are substance-related effects. Also, it was not clear whether the effects, if any, should be considered adverse. Therefore, the full study report (Burbacher *et al.,* 1999) was consulted.

Five methanol-exposed females were caesarean-sectioned due to pregnancy complications (uterine bleedings in 4 females) and prolonged unproductive labour (1 female). Although these complications were not observed in the control group, the findings were not dose-dependent or statistically significant, as the incidences were 2 at the low dose, 2 at the mid dose, and 1 at the high dose (out of 8-9 animals per group).

The mean duration of pregnancy in the methanol-exposed groups was significantly decreased, by 6-8 days when compared to controls. However, there was no dose-response relationship, as the durations of pregnancy were 168, 160, 162, and 162 days in the control, low, medium, and high dose groups, respectively. Furthermore, the duration of pregnancy was within the reported normal range for this species (NTP-CERHR, US NTP 2003).

There were no effects on birth weight, growth or health of the infants. Eight different behavioural tests were conducted, with six of them negative. Infant sensorimotor development was assessed by determining the age when infants successfully reached for and retrieved a small object in full view in order to receive a reward. There were no effects of the methanol exposure on the female infants (34, 33, 28, and 40 days in the control, low, medium, and high dose groups, respectively). However, in males there appeared to be an effect of the methanol exposure, with statistically significant delays in the mid and high dose groups (24, 32, 43, and 40 days). However, it should be noted that the group sizes for the males were 3, 5, 3, and 2 infants in the control, low, medium, and high dose groups, respectively. Thus, this finding should be interpreted with caution.

The other test that possibly indicated an effect was a test for infant recognition memory, where the infant's ability to recognise previously seen stimuli from those that were new was assessed. The testing was conducted using two cohorts (3-4 infants/group), with an effect in one (0.70, 0.61, 0.50 and 0.60 in the control, low, medium, and high dose groups, respectively) but not the other cohort. After combining the cohorts, a statistically significant effect only remained in the mid-dose group and a relationship with exposure to the substance can thus be questioned.

An unexpected finding was that at the age of 1-1.5 years, 2 female offspring out of 7 in the high dose group started to suffer from a wasting syndrome, requiring euthanasia when they reached the age of 20 and 36 months, respectively.

An overall assessment of the monkey studies indicated that methanol may have affected the infants, but that the data were not very robust and clearly not sufficient for classification. Furthermore, there were minimal similarities between the very clear effects noted in rodents and those possibly observed in the monkeys. It is acknowledged that the monkey exposure levels ($\leq$1800 ppm) and exposure time per day (2.5 hours in monkey vs 7 hours in mice), were lower than the LOAEC of 2000 ppm in mice, and the blood methanol concentration was 35 mg/L at the top dose in monkeys when compared to 537 mg/L in mice at the LOAEC. Therefore, developmental toxicity also in monkeys at higher exposure levels cannot be ruled out.

The RAC concludes that there is robust evidence of developmental toxicity of methanol in rodents, but very limited indications of developmental toxicity from non-rodent species which have metabolic pathways more similar to humans. In addition, it is noted that the findings of developmental toxicity in rodents only occur at high exposure levels (with lowest LOAELs/LOAECs of 1000 mg/kg (Youssef, 1997) and 2000 ppm (Rogers, 1993), via the oral and inhalation route, respectively).

Comparison with the criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

There is no indication from human experience of developmental toxicity of methanol, and Category 1A is therefore not appropriate.

Classification in Category 1B is largely based on data from animal studies, providing clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects. . Although methanol causes developmental toxicity in rodents, there are limited indications of developmental effects in non-rodent species having metabolic pathways for methanol more similar to those occurring in humans. The relevance of extrapolating rodent toxicity data to humans can therefore be questioned. Accordingly, the CLP guidance concludes that rat acute toxicity data is of no relevance for humans, because there is human evidence of a much higher acute toxicity of methanol than in rodents, presumably caused by the formic acid that is formed in humans but not in rodents. It is also noted that developmental toxicity in rodents only occurs at high exposure levels, and it is possible that such high exposure levels would generate such high blood concentrations of formic acid in humans that maternal toxicity (acidosis, blindness, lethality) would occur. Taken together, the RAC is of the opinion that the rodent data are not sufficient to presume similar effects in humans, and a classification with Category 1B is therefore not appropriate.

Category 2 is an option when there is some evidence from experimental animals of an adverse effect on development that are not secondary non-specific consequences of other toxic effects. There was clear evidence of developmental toxicity in rodents, whereas the findings from monkeys and rabbits were not sufficient for classification. The mouse appeared to be the most sensitive species to the developmental toxicity of methanol, but it is noted that rodents have a different metabolism of methanol than humans.

A comparison of methanol blood concentrations in humans and rodents was conducted [Ref] with the aim to establish whether methanol concentrations sufficiently high to cause developmental toxicity can arise in humans without simultaneously resulting in acutely toxic formate concentrations (see also the section 'Supplemental Information - In depth analyses by RAC').

It appears that in humans, blood concentrations similar to those seen in mice at inhalation concentrations leading to developmental toxicity findings which clearly meet the classification criteria (cleft palates were observed at 5000 ppm and a blood concentration of 1650 mg methanol/L), would be lethal. Blood concentrations similar to those in the mouse at the LOAEC (increased incidence of cervical rib anomalies) would, in humans, be accompanied by signs of acute methanol intoxication (caused by formate). These signs could be nasal irritation, nausea, blurred vision, and mild CNS depression 6-30 hours later (NAS/COT Subcommittee for AEGLs (2005)) in severe cases, followed by acidosis and impaired vision (blindness). At an exposure level equivalent to the mouse NOAEC (1000 ppm), only slight effects may arise in humans.

If this comparison was conducted using the rat LOAEC for developmental toxicity, such methanol concentrations may be acutely lethal to humans.

There are known differences among individuals and populations with respect to the availability of alcohol dehydrogenase (polymorphism), but there are also different isozymes of alcohol dehydrogenase that contribute to the metabolism of methanol, and additionally other enzymes operating in other steps of the metabolism, making it difficult to predict the overall consequences of enzymatic variations on the overall toxicity of methanol.

The above comparison indicates that methanol blood levels causing clear developmental toxicity in rodents would be acutely **toxic or even lethal to humans. Thus, classification for developmental toxicity seems not relevant. The RAC therefore concludes that, based on the available information, there is not sufficient evidence for classifying methanol for developmental toxicity.**

Supplemental information - In depth analyses by RAC

The metabolic differences between rodents and humans are well known, with formate accumulating in humans (but not in rodents), resulting in acute toxic effects such as acidosis, coma, and blindness in humans but not in rodents. However, the developmental toxicity seems to be mediated by methanol itself, without any contribution from formate (Dorman *et al.*, 1995). A more detailed comparison of methanol blood concentrations in humans and rodents has therefore been conducted, with the aim to find out if methanol concentration sufficiently high to cause developmental toxicity can arise in humans without simultaneously resulting in acutely toxic formate concentrations.

The comparison is based on data from the US NTP-CERHR monograph on the potential human reproductive and developmental effects of methanol (2003) and the US EPA Toxicological review of methanol (US EPA, 2013). The developmental toxicity studies in rodents giving the lowest NOAECs/NOAELs for the inhalation and oral routes, and for which there are also blood methanol measurements, are briefly described below. These concentrations are then compared with blood methanol concentrations measured in human case studies and in controlled chamber studies with human volunteers.

In mice exposed via inhalation (7h/day during GD 6-15), at the NOAEC and LOAEC (1000 ppm and 2000 ppm, respectively) blood concentrations of 97 and 537 mg/L were observed, respectively, when measured 15 minutes after cessation of the 7 hours exposure period (Rogers *et al.*, 1993). In the same study, other mice orally received 4000 mg/kg/day during GD 6-15 (only one dose level was used), resulting in developmental toxicity and a blood concentration of 3856 mg/L.

In rats exposed via inhalation (7h/day during GD 1-19), the NOAECs/LOAECs (5000 ppm/10000 ppm) were observed at blood concentrations of 1000-2170 and 1840-2240 mg/L after cessation of the 7 hours exposure period (Nelson *et al.*, 1985). Only one oral rat study covering GD 6-15 is available, showing developmental toxicity after 2500 mg/kg/day, but no analyses of blood methanol was made in this study (De-Carvalho *et al.*, 1994). The above mentioned rodent studies are listed in Table 1 (below).

Table 1. Compilation of key rodent studies

Species/route	NOAEL (ppm or mg/kg/day)	LOAEL/effect (ppm or mg/kg/day)	Blood methanol concentration (mg/L)	Reference
Mouse inhalation	1000	2000	97 / 537	Rogers *et al.,* 1993
Mouse oral	-	4000*	3856**	Rogers *et al.,* 1993
Rat inhalation	5000	10000	1000-2170 / 1840-2240	Nelson *et al.,* 1985
Rat oral	-	2500*	-	De-Carvalho *et al.*, 1994

*Only one dose was used, giving effects, so it is not clear if it is a "true" LOAEL.

**Animals were dosed with 2000 mg/kg twice daily. Measured 1 hour after giving the second

dose.

'-' = no data

US EPA (2013) states that frank effects in humans such as e.g., blurred vision, blindness, coma, and acidosis begin to occur as blood levels approach 200 mg/L, and 800 mg/L appears to be the threshold for lethality. The American Academy of Clinical Toxicology in their Methanol Guidelines state that blood methanol concentrations below 200 mg/L is usually asymptomatic, whereas concentrations above 500 mg/L indicate serious poisoning (Barceloux *et al.,* 2002). They also conclude that correlating blood methanol concentrations to clinical effects is difficult, for instance because the methanol concentration varies with time and thus depends on the time of sampling, which often is unclear in the case studies. Furthermore, the simultaneous ingestion of ethanol protects against the toxicological effects of methanol, and since the case studies often concerns co-exposure to ethanol, the victims are protected by the ethanol. Case studies reporting blood methanol concentrations are listed in Table 2 (below).

Exposure route	**NOAEL (ppm)**	**LOAEL /effect (ppm)**	**blood methanol concentration (mg/L)**	**Reference**
Inhalation – 4h (chamber study)	20	200 / subclinical nasal irritation	Not measured	Mann *et al.,* 2002, as cited in US EPA (2013)
Inhalation – 4h (chamber study)	-	200 / minimal neurobehavioral effects	6.8	Chuwers *et al.,* 1995; d'Alessandro, 1994, as cited in US EPA (2013)
Inhalation – 4h, 200 ppm (chamber study)	-	Kinetic study, effects not studied	6.5	Osterloh, 1996, as cited in US NTP (2003)
Inhalation – 6h, 200 ppm (chamber study)	-	Kinetic study, effects not studied	7-8	Lee, 1992, as cited in US NTP (2003)
Inhalation – 8h, 800 ppm (chamber study)	-	Kinetic study, effects not studied	30.7	Batterman, 1998, as cited in US NTP (2003)
Inhalation (occupational study)	-	459 ppm/ dimmed vision, nasal irritation	Not measured	Kawai, 1991, as cited in US EPA (2013)
Human case study (ingestion)	-	Ocular deficits, coma, death	360	Rubinstein, 1995, as cited in US EPA (2013)
Human case study (ingestion)	-	Acidosis, visual acuity	1630	Hantson, 1997, as cited in US EPA (2013)
		Comatose	12900	
		Comatose	600	
Human case study (ingestion)	-	Mild acidosis	2300	Hantson, 1997, as cited in US EPA (2013)
Human case study	-	Coma, death	1000	Vara-Castrodeza, 2007, as cited in

(ingestion)				US EPA (2013)
Human case study (ingestion)	-	Blindness	370	Keles, 2007, as cited in US EPA (2013)
Human case study (ingestion)	-	Blurred vision, cerebral edema	860	Fotenot and Pelak, 2002, as cited in US EPA (2013)
Human case study (ingestion)	-	Vegetative state	1272	Kuteifan, 1998, as cited in US EPA (2013)
Human case study (ingestion)	-	Death	3044	Gaul, 1995, as cited in US EPA (2013)
Human case study (ingestion)	-	Death	21	Bynevelt, 2007, as cited in US EPA (2013)
Human case study (dermal & inhalation)	-	Vision loss, coma	200	Adanir, 2005, as cited in US EPA (2013)
Human case study (dermal & inhalation, 2 men)	-	Headache	230, 160	Aufderheide, 1993, as cited in US EPA (2013)
Human case study (inhalation, 7 men)	-	Acidosis but recovered quickly	>240	Bebarta, 2006, as cited in US EPA (2013)

'-' = no data

At low human exposure levels, there are reliable studies showing that 4-6 hours inhalation exposure to 200 ppm result in blood concentrations of 6-8 mg/L. The first signs of minimal effects appear at this exposure level. The EU 8 hours indicative Occupational Exposure Limit (iOEL) value for methanol is also 200 ppm (Directive 2006/15/EC).

A blood concentration of 30.7 mg/L methanol has been measured after 8 hours exposure to 800 ppm. Based on the case studies (Table 2), it seems that severe toxicological effects such as blurred vision, blindness, acidosis and coma can occur from blood levels of approximately 200 mg/L methanol.

The human "effect levels" above (6-8 mg/L for the very first slight signs of effects and 200 mg/L for severe toxicity) can be compared with the mouse and rat blood concentrations of methanol at the inhalation NOAECs (97 and 1000 mg/L, respectively). This comparison shows that at the rat NOAEC, humans would suffer from lethal effects. Exposure of humans resulting in human blood concentrations similar to the mouse NOAEC (97 mg/L) is likely to cause effects in at least some humans.

Several physiologically based pharmacokinetic (PBPK) models have been developed, the latest by US EPA (2013) based on previous models. The PBPK model predicts that a human blood concentration of 97 mg/L will result from inhalation exposure to concentrations of slightly higher than 1000 ppm (US EPA, table B-6). According to Kawai (1991), mean occupational exposure levels of 459 ppm have resulted in dimmed vision and nasal irritation. The modelling and the comparison with the study by Kawai (1991) supports that pregnant women exposed to methanol at concentrations resulting in blood levels similar to the mouse NOAEC will likely be

affected by some signs of acute methanol intoxication. However, there are many uncertainties involved in this assessment, so it is difficult to quantify the potential maternal toxicity.

In humans having a methanol blood concentrations of 537 mg/L, similar to that observed in mice at the LOAEC, the maternal toxicity would be expected to be severe.

As noted above, blood concentrations in humans similar to those seen in mice at inhalation concentrations leading to developmental toxicity findings which clearly meet the classification criteria (5000 ppm -> cleft palates at 1650 mg methanol/L blood) would be lethal.

A similar comparison cannot be made for the oral exposure as the rodent data are rather poor. However, based on the single observation of a blood concentration on 3856 mg/L in mice showing signs of severe developmental toxicity (number of live foetuses decreased by 44%, incidences of malformations greatly increased), it is noted that a similar blood level in humans would be lethal.

There are many uncertainties to consider in this comparison, and an important one is that the proposed human threshold for severe acute toxic effects of 200 mg methanol/L blood is not very robust (see Table 2).

To summarise, it appears that blood concentrations in humans similar to those seen in mice at the inhalation LOAECs would be accompanied by signs of acute methanol intoxication (caused by formate). These signs could be nasal irritation, nausea, and mild CNS depression, later in severe cases followed by impaired vision (blindness) and acidosis. At the mouse NOAEC, only slight effects may arise.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

Not evaluated in this dossier.

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