Committee for Risk Assessment

RAC

Annex 2
Response to comments document (RCOM)
to the Opinion proposing harmonised classification and labelling at EU level of

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

CLH-O-0000004421-84-03/F

Adopted

12 September 2014

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

ECHA accepts no responsibility or liability for the content of this table.

Substance name: Methanol
CAS number: 67-56-1
EC number: 200-659-6
Dossier submitter: Italy

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2013	Germany		MemberState	1
Comment received				
The DE CA does not support the conclusion for the proposal to classify the substance as Repr. 1B – H360D. Based on the data presented in the dossier, classification as Repr. 2 ("Suspected human reproductive toxicant") appears more appropriate instead.				
Dossier Submitter's Response				
Thank you for the comment. Noted.				
RAC's response				
Whereas the rodent toxicity data in isolation clearly fulfils the requirements for Repr. 1B classification, the criteria also require that the data should lead to a strong presumption that the substance has the capacity to interfere with reproduction in humans. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification and that the metabolic differences also have to be considered when deciding on the classification. Based on an overall assessment of the data, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. The RAC therefore argues that methanol should not be classified for developmental toxicity, neither in Category 1B nor Category 2.				

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	Germany		Individual	2
Comment received				
Please find attached as pdf my personal opinion on the classification of Methanol as a reproductive toxicant. *ECHA's note: The information provided in the following attachment :PERSONAL OPINION ON THE CLASSIFICATION OF METHANOL AS DEVELOPMENTAL TOXICANT [attachment 3] was copied in comment no.13*				
Dossier Submitter's Response				
MeOH consistently induced developmental effects in the absence of maternal toxicity in *in*				

vivo studies on rodent and non-rodent laboratory animal species (rats, mice, rabbit and monkey) performed by the most relevant route of exposure (inhalation).
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. The findings of human studies are too limited to allow a conclusion concerning the developmental toxicity of MeOH. Therefore the assessment of MeOH developmental toxicity should rely on experimental data. Accordingly, our proposal for classification is based on the added value of weight of evidence, as provided by the integrated assessment of the available experimental studies. In particular MeOH produces severe developmental effects in rats, mice, rabbit and monkey in absence of maternal toxicity; such developmental effects include, but not limited to, teratogenicity in both rodent species as well as in rabbits. Moreover MeOH produce placental adverse effects in rats and mice *in vivo* and in human placental explants *ex vivo*.
It is acknowledged that MeOH has a considerable acute toxicity in humans; however the avalable data cannot allow to conclude that such acute toxicity would prevent the onset of developmental effects at equal or lower exposure levels.
The mechanisms underlying the developmental effects of MeOH in rodents and in rabbit involve several modes of action, including the generation of reactive oxygen species, the interference with placental functions and the production of formaldehyde by a folate-dependent pathway. There is no evidence that such mechanisms might be not relevant to humans. Moreover, the ability to metabolize MeOH, as well as the vulnerability to reactive oxygens species may show a considerable variability in humans as a result of genetic and environmental factors; therefore the available data suggeast that some subjects and/or subgroups could be higly susceptible to MeOH developmental toxicity.
Based on the above considerations, the classification of MeOH as developmental toxicant Repr.1B – H360D is regarded as both adequately conservative and scientifically justified.

RAC's response

See response to comment no. 13.

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	Italy	IReS	Industry or trade association	3

Comment received

Data and information presented by Italian Member State Competent Authority in order to classify methanol as reprotoxic substance (cat. 1B) are mainly based on observation in animals. However, the overall assessment did not take into account the profound difference existing between human and animal toxicokinetics. This difference may arise questions on the relevance for humans of the observed effects, according to Annex I (3.7.2.3.2.) of CLP Regulation, where it is clearly stated that toxicokinetic differences are relevant for establishing the proper level of concern.

Dossier Submitter's Response

Thank for the comment.
We agree that the major observations are on animals but, the findings of human studies are too limited to allow a conclusion concerning the developmental toxicity of MeOH. Therefore the assessment of MeOH developmental toxicity should rely on experimental data.
While we agree that the mechanisms underlying the developmental effects of MeOH in rodents and in rabbit involve several modes of action, it should be noted that there can be a considerable variability in humans as a result of genetic and environmental factors. in fact the available data suggest that some subjects and/or subgroups could be higly susceptible to MeOH developmental toxicity.
Thus the classification of MeOH as developmental toxicant Repr.1B – H360D is regarded as

both adequately conservative and scientifically justified.
RAC's response
Whereas the rodent toxicity data in isolation clearly fulfils the requirements for Repr. 1B classification, the criteria also require that the data should lead to a strong presumption that the substance has the capacity to interfere with reproduction in humans. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC agrees with the comment that the rodent toxicity data cannot be used in isolation for the classification and that the metabolic differences also have to be considered when deciding on the classification. Based on an overall assessment of the data, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. The RAC therefore argues that methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	France		MemberState	4
Comment received				
Despite the fact that the CLH report would not be updated, it would have been useful to have the number of animals used for each study reported. We note that most of the studies were performed at pretty high doses (g/kg bw), nevertheless clear effects on development have been observed in absence of maternal toxicity. Therefore, FR supports the proposed classification for human health Repr.1B – H360D.				
Dossier Submitter's Response				
Thank you for the comment.				
RAC's response				
In order to decide whether the the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. The RAC also notes the high dose levels in the positive rodent studies. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.				

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	Hungary		MemberState	5
Comment received				
According to the data presented in the dossier, it seems, there is a difference in the sensibility of rodents and other species like humans against methanol. However, the experimental data with several species altogether provide clear evidence of prenatal developmental toxicity induced by methanol. Consequently, on the basis of the published results, the Hungarian REACh-CLP CA agrees on the necessity to classify methanol as				

Repro. Cat. 1B, H360D.
Dossier Submitter's Response
Thank you for the comment.
RAC's response
In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
20.11.2013	France		Individual	6
Comment received				
L'Italie propose d'ajouter la toxicité pour la reproduction à la classification existante. Cette proposition est basée sur des preuves scientifiques selon lesquelles le méthanol a des effets néfastes sur le développement de l'enfant à naître : je soutiens cette proposition. ECHA note: The last sentence was removed from this comment because the content was considered not relevant to the CLH process.				
Dossier Submitter's Response				
Thank you for the comment.				
RAC's response				
The support is noted. However, even though the toxicological effects in the rodents are scientifically robust, the criteria require that there is a strong presumption that methanol also can affect reproduction in humans. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. The RAC is of the opinion that the high acute toxicity of methanol in humans will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.				

Date	Country	Organisation	Type of Organisation	Comment number
12.12.2013	Belgium	Methanol Institute	Industry or trade association	7
Comment received				
Please see attached comments. *ECHA's note: The information below was provided in the following attachment: Methanol Institute Comments on the Proposed Classification of Methanol as a Reproductive Toxicant Under the CLP Regulation [attachment 2]* **Overview**				

Italy is proposing that methanol be classified as a Category 1B hazard: potential human developmental toxicant based on animal studies. The developmental studies of methanol in animals should not be used as the basis for labeling methanol because of species differences in metabolism, achievable blood levels of methanol, and the mode of action of developmental effects in rodents.

Classification based on rat and mouse studies would critically miss the specific acute toxicity of methanol in humans, which includes blindness. Such high acute toxicity is not seen in rodents.

Effects seen in rats and mice for developmental toxicity occur at very high exposure levels, exceeding oral limit doses or their equivalent in inhalation exposures advised in OECD guideline studies; levels that would be lethal to humans. Therefore, the developmental effects in rats and mice are not relevant to human hazard assessment and should not be the basis for classification.

Metabolic Differences

Rodents and non-rodent species metabolize methanol to formaldehyde to formic acid to carbondioxide. There are two major differences, however. Rodents (mice and rats) metabolize methanol to formaldehyde using the enzyme catalase, whereas humans use alcohol dehydrogenase (ADH1). A byproduct of rodent methanol metabolism is hydrogen peroxide, a reactive oxygen species (ROS). ROS may be involved in the mode of action for methanol-induced adverse developmental effects in rodents. Secondly, in humans the conversion of formic acid to carbondioxide is rate limited because humans have much lower levels of folate, a required component of this metabolic step, resulting in formic acid accumulation and toxicity. Thus, exposure to high levels of methanol result in increased ROS in rodents, and formic acid in humans.

Formic acid produces toxicity to the optic nerve, resulting in blindness in humans exposed to high levels of methanol. Secondly, the increased formic acid causes a metabolic acidosis, which can be lethal if untreated. Thus, the lethal dose of methanol in humans is estimated to be between 300 and 1000 mg/kg, which is ***lower*** than the dose that causes developmental effects in rodents.

The Italian CLH dossier suggests that polymorphisms in certain populations may give greater susceptibility to reduced methanol clearance. However, humans with alcohol dehydrogenase (ADH1) allele variations ADH1B*2 and ADH1B*3 frequently seen in Asian populations would tend to metabolize methanol more quickly than populations with ADH1B*1 (based on investigations by Hurley & Edenberg, 2012 and Chen et al., 2009 compared to enzyme kinetics for methanol reported by Lee et al., 2011). Differences in acetaldehyde dehydrogenase (ALDH) are not expected to impact methanol metabolism or formate metabolism because, as noted in the CLH dossier, ALDH does not compete with formaldehyde dehydrogenase (ADH3). Whereas polymorphisms are frequent to ALDH, ADH3 is characterized by monomorphism (Benkmann et al., 1991). These allele variations of ADH1 and ALDH in populations are therefore not expected to impact the human hazard assessment of methanol.

Blood Level Associated with Developmental Toxicity

Developmental toxicity in rodents has been reported from high inhalation or oral exposures to methanol. Measurement of blood methanol during these exposures demonstrates that methanol causes developmental effects in rodents when the blood level of methanol is greater than 537 mg/L (NTP, 2003). Exposure of humans to 800 ppm (~133 mg/kg) for 8

hours resulted in a blood level of 31 mg/L (Batterman et al., 1998). Thus, it is unlikely that humans can be exposed to sufficient methanol to result in a blood level even approaching 500 mg/L and still survive.

Mode of Action for Developmental Effects

Similar to humans, rabbits metabolize methanol to formaldehyde using the alcohol dehydrogenase system, and in the subsequent metabolism of formaldehyde exhibit a greater accumulation of formic acid than occurs in rodents. For these reasons, studies with rabbits are considered more relevant to the human health hazard assessment of methanol than rodents (Sweeting et al., 2011, 2010). A preliminary investigation of the teratogenic potential of methanol in rabbits by Sweeting et al. (2011) reports ***no*** statistically significant developmental effects from the near lethal dose level of 4000 mg/kg bw/d (ip) on days 7 or 8 of gestation in the screening study. A few abnormalities were found among fetuses of treated rabbits (open posterior neuropore in addition to tail abnormalities (2 foetuses in one litter), abdominal wall defect (one foetus), frontal nasal hypoplasia (3 foetuses) and a few with short or missing tail). None of these incidences were statistically different from control rabbits and do not represent evidence of developmental effects, as they could be caused by delayed development because of maternal or fetal toxicity.

Catalase activity at birth in humans is about 10% of the level in adults, while catalase activity in mice at the embryonic stage is about 5% of the adult level. Catalase is necessary for the detoxification of reactive oxygen species produced by a variety of reactions in animals. The protective role of catalase has been demonstrated in a study (Miller and Wells, 2011) using whole embryos in culture; the authors demonstrated that developmental effects from methanol increased when catalase was removed by genetic engineering. Methanol causes greater developmental effects in acatalasemic mice (no catalase) than in wild-type catalase-normal mice. Furthermore, mice expressing high levels of human catalase (hCat), were protected from developmental effects of methanol.

MeOH induces the *in vivo* expression of embryonic NADPH oxidases (**NOXs**), which produce superoxide that subsequently forms hydrogen peroxide and hydroxyl radicals. This enhanced NOX expression together with a reduction in MeOH embryopathies in culture by pretreatment with a NOX inhibitor suggest that MeOH-enhanced embryonic NOX expression and ROS production play an important role in the mechanism of MeOH teratogenesis. It is not known whether MeOH enhances embryonic NOX expression in rabbits, but if not, this could account for the observed species differences in teratological susceptibility.

A recent publication suggests that depletion of glutathione (GSH) caused by methanol metabolism in rodents may play a role in the developmental toxicity of methanol in rodents (Siu et al., 2013). This has not been tested in rabbits.

Conclusions

At doses that are lethal in humans, New Zealand white rabbits are almost completely resistant to MeOH teratogenicity, although it is possible that broader exposure throughout gestation may yield defects. Rodents, in contrast, are variably resistant or sensitive to MeOH teratogenicity, depending upon the strain. Sensitive rodent strains exhibit numerous birth defects that differ by strain, and these defects include a broad spectrum of skeletal malformations. There are a number of lines of evidence for the involvement of ROS in the mechanism of rodent MeOH teratogenesis, although conflicting results have been published, and embryonic formation of formaldehyde also may play a teratogenic role. The mechanism underlying the resistance of rabbits to MeOH teratogenicity has not been determined. Overall, rabbits and several strains of mice and rats are highly resistant to MeOH

teratogenesis, and it is not clear that human risk can be accurately estimated by results from sensitive rodent strains.

References:

Batterman S.A., Franzblau A., D'Arcy J.B., Sargent N.E. Gross K.B., Schreck R.M. (1998). Breath,urine, and blood measurements as biological exposure indices of short-term inhalation exposure to methanol. Int Arch Occup Environ Health 71:325-35.

Benkmann H.G., Agarwal D.P., Saha N., and Goedde H.W. (1991)Monomorphism of formaldehyde dehydrogenase in different populations. Hum Hered, 41, 276-8.

Chen Y.C., Peng G.S., Wang M.F., Tsao T.P., and Yin S.J. (2009) Polymorphism of ethanolmetabolism genes and alcoholism: Correlation of allelic variations with the pharmacokinetic and pharmacodynamic consequences. Chemico-Biological Interactions, 178; 2-7.

Hurley, T.D. & Edenberg, H.J. (2012) Genes encoding enzymes involved in ethanol metabolism. Alcohol Research: Current Reviews, 34(3), 339-344.

Lee S.L., Shih H.T., Chi Y.C., Li Y.P., Yin S.J. (2011) Oxidation of methanol, ethylene glycol, and isopropanol with human alcohol dehydrogenases and the inhibition by ethanol and 4-methylpyrazole. Chem Biol Interact,191,26-31.

Siu M.T., Shapiro A.M., Wiley M.J., Wells, P.G. (2013). A role for glutathione, independent of oxidative stress, in the developmental toxicity of methanol. Toxicol. Appl. Pharmacol. http://dx.doi.org/10.1016/j.taap.2013.09.020.

[ECHA note: End of attachment 2]

Dossier Submitter's Response

The avalable data cannot allow to conclude that the marked acute toxicity of MeOH in humans would prevent the onset of developmental effects at equal or lower exposure levels. Thus, classification should be based on experimental data that show developmental toxicity in the absence of maternal toxicity in two rodent and two non-rodent species. The mechanisms underlying the developmental effects of MeOH involve several modes of action, including the generation of reactive oxygen species, the interference with placental functions and the production of formaldehyde by a folate-dependent pathway. There is no evidence that such mechanisms might be not relevant to humans. Moreover, the suscebtibility to such toxicity mechanisms may show a considerable variability in humans as a result of genetic and environmental factors; accordingly, some subjects and/or subgroups could be higly susceptible to MeOH developmental toxicity.

Based on the above considerations, the classification of MeOH as developmental toxicant cat Repr. 1B – H360D is regarded as both adequately conservative and scientifically jiustified.

RAC's response

In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans

(via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
11.12.2013	Germany	REACH Methanol Consortium	Industry or trade association	8

Comment received

See attached public attachment

ECHA's note: The information provided in the following attachment: Consultation C&L Methanol [attachment 1] was copied in comment no. 11.

Dossier Submitter's Response

Thank you for the comment. A detailed reply is provided at comment number 2.

RAC's response

See response to comment number 11.

Date	Country	Organisation	Type of Organisation	Comment number
10.12.2013	Belgium	Cefic	Industry or trade association	9

Comment received

Critical points to be made in opposing the proposal for classification of methanol as Repr. 1B – H360D:
The basis of our opposition to the classification of methanol for developmental toxicity lies on three different aspects of the CLH Report. First, the discussion of metabolism and kinetics with which we essentially agree; second, the description of the developmental toxicity studies which we think omits critical data, and misinterprets the results of rabbit (Sweeting et al., 2011) and primate studies (Burbacher et al., 2004); and third, the differences in general toxicity of methanol between rodents and primates which has barely been discussed in the CLH Report.

1.Toxicokinetics and metabolism:

We agree with the review of the toxicokinetics in the CLH Report, section 4.1, which clearly defines the differences in the metabolism of methanol in rodents compared with that in humans and other primates. It also states that the metabolism in rabbits, more accurately than rodents, reflects primate methanol and formic acid kinetic profiles. In rodents the rate limiting step in metabolism is Step 1, which is the conversion of methanol to formaldehyde. In primates the rate limiting step is Step 3, the conversion of formate to CO2 and water. The consequence of this is that administration of high doses of methanol to rodents results in high circulating levels of methanol, whereas in humans, methanol levels rapidly decline and high levels of formate accumulate.

In discussing Step 2, the conversion of formaldehyde to formate, the CLH report states that in all species this is very rapid (half-life ~1 minute), and formaldehyde does not accumulate in animals or humans exposed to methanol.

An important difference between rodents and primates is further described in the kinetics section of the CLH Report which relates to Step 3 of methanol metabolism which is the conversion of formate to CO2 and water. In rodents this conversion is carried out through

two enzyme systems; one folate dependent and one catalase dependent, and is very rapid. In contrast, in primates, only one system is used which is the folate dependent one, in which formate combines with tetrahydrofolate (THF), and the levels of THF in primates is much lower than in rodents, so the conversion is less efficient. The CLH Report also reports that in mice there may be a third or more systems capable of oxidising formate to CO2 which may operate at different formate tissue levels, and that primates do not have such systems available, making primates more sensitive to formate induced metabolic acidosis following methanol exposure.

The CLH Report concludes in its review of formic acid as a toxic metabolite of methanol leading to acidosis, that "formic acid accumulation occurs in human, rabbit and primates but not in rodents." It continues, "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." This is of importance in considering the general toxicity and lethality of methanol poisoning, but is not relevant to developmental toxicity since it has been clearly shown that formate is not a developmental toxicant in rodents or rabbits (discussed later).

To conclude, we feel that the major differences in metabolism between rodents (mice and rats) on the one hand resulting in high circulating methanol levels, and rabbits, primates and humans on the other hand, resulting in high circulating formate levels, means that one cannot have a "strong presumption" that the results of developmental toxicity studies in rodents can be applied directly to humans, as is required under the CLP Regulation for classification to be applied.

2. Developmental toxicity:

Rodents:

We agree that the summary of the developmental toxicity as shown in Table 1 of the CLH Report, shows clearly that in mice and rats, exposure to high doses of methanol, usually greater than 1g/kg bodyweight, results in a high number of congenital malformations affecting primarily the CNS, ocular and skeletal systems.

Rabbits:

Only one study in rabbits is reported (Sweeting et al., 2011), which is part of a larger study comparing metabolism and teratogenicity of methanol in NZW rabbits and 3 mouse strains. Only 5 control and 10 treated litters were evaluated and the methanol was injected intraperitoneally on one day only (Day 7 or 8 of gestation) in a total dose of 4g/kg bodyweight. The description of the study in Table 1 is incomplete since it mentions tail and other abnormalities in the treated fetuses, but does not mention that none of these malformations were statistically significantly different from the controls.

We therefore do not agree with the Summary and Discussion Section 4.12.1 review of this study which states that the rabbit study "showed an increase of malformations, mainly tail abnormalities, without overt signs of maternal toxicity. Therefore, the study suggests that MeOH may act as a teratogen also in non-rodents." Since no statistically significant differences were found in this study in incidences in fetal resorptions, stillbirths or postpartum lethality, fetal weights or fetal malformations, one cannot state that it suggests that methanol may be a teratogen in the rabbit.

Non-human primates:

One 2 cohort study in monkeys is also reviewed (Burbacher et al., 2004). This was a fertility and postnatal developmental toxicity study which extended over several years. Animals were treated with methanol before and during mating and gestation, with no methanol treatment of the offspring postnatally. We agree with the summary presented in Table 2 of the CLH Report, but in the first paragraph where it mentions that the mean length of pregnancy was significantly decreased by 6-8 days compared with controls, it should have added that the decrease was not dose related with the shortest mean duration of 160 days being in the lowest dose group, 162 and 162 days in the mid and high dose groups, compared with 168 days in controls. This suggests that the small differences in pregnancy duration are not treatment related.

The discussion of this study in Section 4.12.1 is very brief suggests that the reduction in pregnancy duration and the presence of pregnancy complications at all exposure levels, without significant differences between levels, shows that "a NOAEC was not identified." This is misleading since the paper clearly states that the incidence of pregnancy complications was not significantly increased (P=0.24), and since the reduction in duration was not dose related it was probably not treatment related. Thus, the highest dose level of 1800 ppm can be regarded as a NOAEC. The CLH discussion does not mention that there were no effects of treatment on menstrual cycles or fertility, and no other signs of developmental toxicity were observed with no effects on fetal survival, birth weight, crown-rump length, head circumference, head length and width, and no increase in congenital malformations. In addition, as mentioned in the CLH Report, there was no clear evidence of effects on neurobehavioural development in the study of the offspring.

Humans:

We agree with the conclusion of the CLH Report that the findings in humans are inconclusive concerning the developmental toxicity of methanol.

In Conclusion:

We agree that there is clear evidence for developmental toxicity in rodents. However, one (limited) study in rabbits did not show any significant developmental toxicity at a dose level of 4g/kg bodyweight methanol as a single dose on gestation day 7 or 8. In addition, one extensive study in monkeys at inhalation dose levels up to 1800 ppm daily for almost one year did not show any maternal toxicity or effects on menstrual cycles, fertility or embryofetal development or postnatal behavioural development.
Therefore we do not agree with the following paragraphs in the Summary of MeOH developmental effects, Section 4.13, page 46 of the CLH Report:
"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." We do not agree, because there was no significant increase in teratogenic effects in this rabbit study.

Also the paragraph "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." We do not agree because this effect was not clearly treatment related, and there were no adverse effects at any dose level on fetal development or increase in malformations in primates.

3. General toxicity of methanol

The differences in metabolism and kinetics and other factors result in a very significant

difference in the general toxicity of methanol in rodents and humans. In humans methanol toxicity is manifest as CNS depression, followed later by metabolic acidosis and ocular toxicity ranging from blurred vision up to complete blindness. Such effects can result from ingestion of as little as 4 ml (60 mg/kg bw) methanol (IPCS, 2001). The minimal lethal dose in humans is 300 mg/kg bw (IPCS, 1997). This can be contrasted with rodents which can tolerate up to 5 g/kg bw daily without adverse effects, as reviewed in the CLH Report. The toxicity in humans is largely thought to be related to the high levels of formic acid produced from methanol, and a formic acid level around 0.5g/l in blood is a good indicator of potential lethality in poisoning cases (Ferrari et al., 2003).
The importance of these observations is that in pregnant rodents, it is possible to administer very high dose levels of methanol, greater than 1 g/kg bw per day, which are associated with developmental toxicity and malformations, with no maternal toxicity. Such dose levels would be lethal in humans. Although considerable efforts have been made to identify the active substance causing malformations in rodents (see the review of in vitro studies in the CLP Report) we do not know what the proximal teratogen of methanol is in rodents. It is not formate since standard OECD 414 developmental toxicity studies in rats and rabbits up to 945-1000 mg/kg bw did not show maternal or developmental toxicity (ECHA Reach Registration Dossier on Sodium Formate, 2012). It is possible that free methanol, reactive oxygen species (ROS) and rodent embryonic catalase may be determinants of teratogenic risk (see CLH Report page 35). All of this suggests that effects at high doses in rodents may be of no relevance to humans, since general toxicity and lethality would prevent such a sequence of events.

This is exactly analogous, but opposite, to the situation in the classification of methanol for Acute Toxicity Cat 3. In Guidance on the Application of the CLP Criteria Version 4, 2013, Section 3.1.6.1.1 the example for methanol is given, with the rationale for not classifying based on the Animal data: "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)" For the Human data it states "The minimum lethal dose reported of 300 mg/kg bw is used as equivalent ATE; according to CLP Annex I, Table 3.1.1 the resulting classification is Category 3."

Similarly for the Classification for STOT-SE in Section 3.8.6.1.1 for methanol the rationale for not classifying based on animal data is the same as above, and for the human data rationale is: "The classification criteria for Category 1 are fulfilled: clear human evidence of a specific target organ toxicity effect which is not covered by Acute toxicity."

In Conclusion:

We do not agree with the Conclusion for Classification of MeOH in Section 4.15 of the CLH Report that methanol should be classified Repr. 1B – H360D: In our opinion, the same type of reasoning that has been used in classifying methanol for Acute toxicity and for STOT-SE, but in reverse, should be applied to consideration of the data for developmental toxicity. The clear data for methanol induced teratogenesis in rodents at high dose levels, is not considered to be a good model for human effects. The data are not relevant for classification in humans since primate data and supporting rabbit data have not demonstrated teratogenic effects, and it is not possible to expose primates and humans to such high dose levels as rodents. We therefore propose that methanol should not be classified for developmental toxicity as was previously agreed by the Classification Committee under the Dangerous Substances Directive.

References
Burbacher T.M., Grant K.S., Shen D.D., Sheppard L., Damian D., Ellis S. and Liberato N. (2004) Chronic maternal methanol inhalation in nonhuman primates (Macaca fascicularis):

reproductive performance and birth outcome, Neurotoxicology and Teratology 26(5) 639-650.
Ferrari L.A., Arado M.G., Nardo C.A. and Giannuzzi L. (2003) Post-mortem analysis of formic acid disposition in acute methanol intoxication, Forensic Science International 133(1-2) 152-158.
International Programme on Chemical Safety (IPCS) (2001) Methanol, Poisons Information Monograph, PIM 335, World Health Organisation, Geneva.
International Programme on Chemical Safety (IPCS) (1997) Methanol, Environmental Health Criteria 196, World Health Organisation, Geneva.
Sweeting J.N., Siu M., Wiley M.J. and Wells P.G. (2011) Species- and strain-dependent teratogenicity of methanol in rabbits and mice, Reproductive Toxicology 31(1) 50-58.

Dossier Submitter's Response

Thank you for the comment. Please refer to the response to comment number 2

RAC's response

The RAC also notes that the toxicological effects in rabbits and primates are not very robust, and clearly not sufficient for classification. The RAC has evaluated the metabolic differences between rodents and humans, and come to the conclusion that the species differences are too big to allow using the rodent toxicity data as such for classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, it seems very likely that the high acute toxicity of methanol in humans will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	France		MemberState	10

Comment received

p.44 section 4.12.1: It could be useful to add the range of doses used in the studies in order to know which doses are considered as "high doses".

Dossier Submitter's Response

Thank you for the comment. We agree.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.12.2013	Germany	REACH Methanol Consortium	Industry or trade association	11

Comment received

See attached public attachment

ECHA's note: The information below was provided in the following attachment: Consultation C&L Methanol [attachment 1]

This document is submitted by the lead registrant, BASF SE on behalf of the Methanol

REACH Consortium
Date: 10/12/2013
RE: ECHA Consultation period 29/10/2013 to 13/12/2013 on Harmonised Classification and Labelling - Methanol (CAS nr 67-56-1; EC nr 200-659-6)

Methanol REACH Consortium Comments on the Proposal for the Classification of Methanol as a Reproductive Toxicant under the CLP Regulation

Summary

The Methanol REACH Consortium disagrees with the proposal to classify methanol for Reproductive Toxicity category 1B as the criteria for such a classification set forth in the CLP Regulation are not met. Based on the available evidence humans are not susceptible to the developmental toxicity observed in rats and mice, due to differences in metabolism. Therefore, the criterion for *"data which provide a strong presumption that the substance has the capacity to interfere with reproduction in humans"* has not been met. Moreover, it is clear from the available animal data that, based on the differences in metabolism and the formation of formic acid in humans which leads to maternal toxicity at much lower concentrations, the developmental effects observed in rats and mice in the absence of maternal toxicity are not relevant to humans.

Methanol is already used in the ECHA Guidance on CLP as an example for not using rodent toxicity data to classify methanol for acute toxicity and specific organ toxicity on the basis of the non-relevance of rodent toxicity data to humans. This is due to species differences between humans and rodents, rendering the rodent data on methanol irrelevant to humans. The same approach should be applied for developmental toxicity.

The Italian CLH dossier does not recognise the species-dependent observed developmental toxicity in rodents because it does not compare blood methanol or blood formate levels, misinterprets data from a rabbit study, and does not consider the section of the REACH registration dossier on the uniquely high acute toxicity of methanol in humans.

The Italian CLH dossier references a previous review of methanol by the Health Council of the Netherlands, but does not adequately consider the context and data used at that time. The CLH dossier quotes from a 2006 report of the Dutch Council, although citing a more recent 2010 report. Compared to the 2006 report, the Council's review in 2010 actually highlights species differences and the limited relevance that methanol developmental toxicity in rodents has to humans.

Methanol has a high acute toxicity for humans with target organ toxicity for the ophthalmic nerve, which is different to toxicity seen in rodents. There is a large database on the toxicity of methanol and more recent data further supports the existing EU decision not to classify methanol for developmental toxicity under Directive 67/548/EEC.

The authors of the CLH dossier base their deliberations, as they say, *"on an added value of weight of evidence"* and not upon a convincing key study. Many poor evidences, however, from many investigations, cannot build up to strong evidence. In contrast, the total evidence for a possible relevance of effects observed in rodents to humans remains very poor and does by no means suffice for such a classification. None of the developmental studies are really conclusive for a cat.1 classification.

Scientific and Regulatory Analysis

Methanol is a developmental toxicant in rodents but humans metabolise methanol

differently, which is the basis for not classifying methanol for developmental toxicity in humans. Rodents oxidise methanol by catalase, whereas methanol oxidation occurs in humans by alcohol dehydrogenase[1].

There is an increasing database that gives evidence that developmental toxicity in rodents results from the role of catalase. Moreover, blood methanol levels of around 540 mg/L in mice, the lowest levels at which developmental toxicity has been observed in rodents, are not relevant to human health hazard assessment because:

- saturation of the methanol oxidation pathway already occurs in mice at the corresponding exposure of 2000 ppm (inhalation) but not humans;

- severe acute toxicity, including vision loss and potential lethality, from acidosis is associated with blood methanol levels of 540 mg/L in humans but not rodents.

For hazard assessment under the CLP Regulation and the previous Directive 67/548/EEC, dose must be considered together with metabolic, toxicokinetic and other species differences. Under Directive 67/548/EEC Member State experts in the Commission Working Group on Classification and Labelling agreed to not classify methanol for developmental toxicity in humans.

[1] The Toxicology of Methanol, edited by J.J. Clary (2013) provides a source for references.

In 2010, a report from the Health Council of the Netherlands considered metabolism and toxicokinetic differences between rodents and humans to conclude that *"based on the methanol levels measured in the blood of mice and rats… the committee is of the opinion that methanol is not likely to induce reproduction toxic effects in occupationally exposed workers"* (HC, 2010). By contrast, the implications of species differences in metabolism and toxicokinetics to blood methanol levels were not examined in the earlier Dutch Health Council report from 2006 that proposed a classification for methanol as a developmental toxicant (HC, 2006). Recent studies in toxicokinetics of methanol metabolism further characterise the marked species differences (Sweeting *et al*., 2010) and lend supporting evidence to the 2010 conclusion from the Dutch Health Council rather than the proposed classification from 2006.

The Italian CLH dossier puts an emphasis on evidence of potential developmental toxicity observed in primates and rabbits, however these studies clearly demonstrate that there are no equivalent effects as in rodents. With regards to the cited study in primates, the US Health Effects Institute research report (HEI, 1999) concludes: *"Overall, the results provide no evidence of a robust effect of prenatal methanol exposure on the neurobehavioral development of nonhuman primate infants during the first nine months of life."*

In the cited screening study for developmental toxicity in rabbits, the researchers did not find any statistically significant developmental effects (Sweeting et al., 2011). The CLH dossier omits the background rationale and design of this screening study, which was to screen for fundamental species differences with rodents. In this respect, the outcome of the study demonstrates a difference. In particular characteristic traits of methanol developmental toxicity effects of exencephaly, cleft palate, eye malformations observed in rodent studies were not seen in the rabbit. Considering the exposure route (i.p), very high dose level and common variations observed in this rabbit screening study, the study is indicative of species differences but not relevant for drawing a conclusion on developmental toxicity classification.

When considering the complete database available, evidence from animal studies does not

give a strong presumption that methanol has the capacity to interfere with reproduction in humans, a criterion for classification under the CLP Regulation. Human-exposure data do not show an association between methanol exposure and developmental toxicity, another criterion under CLP. However, as a result of metabolic acidosis, methanol is acutely toxic to humans and has a specific toxicity to the ophthalmic nerve.

The Italian CLH proposal for the classification of methanol as a reproductive toxicant category for developmental toxicity under the CLP Regulation therefore has three major shortcomings:

(i) it is not consistent with information on the interspecies differences of methanol toxicity and developmental toxicity of methanol;

(ii) it is not consistent with comparisons of acute toxicity in humans with dose levels required to cause developmental toxicity in rodents;

(iii) it does not follow CLP classification rules.

Interspecies differences of methanol toxicity and developmental toxicity of methanol

Methanol is significantly more acutely toxic to humans than animals[2], which appears linked to the particularly high rate of formic acid formation in humans[3]. In particular, acidosis and ophthalmologic changes are effects in humans that do not occur in rodents or rabbits[4]. But the potential role of formic acid as the ultimate toxic metabolite of methanol is far from clear.

[2] The difference in lethal methanol doses between species is well-established, such as lethal doses in rats and rabbits being 2-3 times higher than those in monkeys, which in turn are 6-10 times higher than the lethal doses reported for humans (NTP, 2003).

[3] The role of formate in methanol-induced toxicity in humans is postulated, but has not been strictly confirmed. For instance, it may be an intermediate or a particular consequence of the metabolic process that gives rise to the toxicity of methanol in humans.

[4] Potential for accumulation of formic acid during the metabolism of methanol in primates is however more closely reflected in rabbits than rodents.
Methanol is classified under CLP for Acute Toxicity category 3 and Specific Target Organ Toxicity, single exposure, category 1. A classification based on rodent studies alone would not yield this classification.

With regards to developmental toxicity, methanol has been shown to cause developmental effects in rats and mice at high dose levels. Compared to humans, rodents metabolize methanol very slowly, resulting in high blood methanol concentrations after dosing. Developmental toxicity in mice is observed at exposure conditions at which the metabolic capacity for methanol is exceeded in rodents (Perkins *et al*., 1995). Although the oxidation pathway differs between primates and rodents (with the alcohol dehydrogenase system in primates versus the catalase system in rodents), it is the subsequent rate of formate oxidation that results in different levels of formate in blood following exposure to methanol.

Developmental toxicity in rodents is not related to the metabolite formate/formic acid. An OECD 414 study investigating sodium formate toxicity in rats at dose levels up to 945 mg/kg bw/d showed no adverse findings in dams and fetuses (ECHA, 2012). A separate OECD 414 study with sodium formate in rabbits at doses up to 1000 mg/kg bw/d also showed no maternal or prenatal developmental toxicity (ECHA, 2012).

Potential for developmental toxicity in rabbits:

Similar to humans, rabbits metabolise methanol to formic acid using the alcohol dehydrogenase system, and exhibit a greater accumulation of formic acid than occurs in rodents. For these reasons, studies with rabbits are considered more relevant to the human health hazard assessment of methanol than rodents (Sweeting *et al*., 2011, 2010). A preliminary investigation of the teratogenicity potential of methanol in rabbits (Sweeting *et al*., 2011) reports no statistically significant developmental effects with two i.p. doses of 2000 mg/kg bw in a screening study.

The description of the study in Table 1 of the CLH dossier is incomplete since it mentions tail and other abnormalities in the treated foetuses, but does not mention that none of these malformations were statistically significantly different from the controls. The Methanol REACH Consortium therefore does not agree with the Summary and Discussion Section 4.12.1 review of this study in the CLH dossier which states that the rabbit study *"showed an increase of malformations, mainly tail abnormalities, without overt signs of maternal toxicity. Therefore, the study suggests that MeOH may act as a teratogen also in non-rodents."* Since no statistically significant differences were found in this study in incidences in foetal resorptions, stillbirths or postpartum lethality, foetal weights or foetal malformations, one cannot state that it suggests that methanol may be a teratogen in the rabbit.

Potential for developmental toxicity in primates:

A two-cohort study in monkeys is reviewed in the CLH dossier which examined fertility and postnatal developmental toxicity over several years. Animals were treated with methanol before and during mating and gestation, with no methanol treatment of the offspring postnatally. The summary presented in Table 2 of the CLH Report should have specified that although the mean length of pregnancy was significantly decreased by 6-8 days compared with controls, that the decrease was not dose related with the shortest mean duration of 160 days being in the lowest dose group, 162 and 162 days in the mid and high dose groups, compared with 168 days in controls. This suggests that the small differences in pregnancy duration are not treatment related.

The discussion of the study in Section 4.12.1 of the CLH dossier suggests that the reduction in pregnancy duration and the presence of pregnancy complications at all exposure levels, without significant differences between levels, shows that *"a NOAEC was not identified."* This is misleading since the paper clearly states that the incidence of pregnancy complications was not significantly increased (P=0.24), and since the reduction in duration was not dose related it was probably not treatment related. Thus, the highest dose level of 1800 ppm can be regarded as a NOAEC. The CLH discussion does not mention that there were no effects of treatment on menstrual cycles or fertility, and no other signs of developmental toxicity were observed with no effects on fo:

Role of catalase in developmental toxicity in rodents:

The role of catalase in the metabolism of methanol in rodents may also be important (Siu *et al*., 2013; MacAllister *et al*., 2011; McCallum *et al*., 2011; Miller & Wells, 2011). A mechanism involving catalase does not have a relevance to humans, due to the different metabolism for methanol when compared to rodents.

This recent research therefore offers further supporting evidence that the developmental toxicity in rodents following exposure to methanol is of limited relevance to humans and supports the conclusion on classification in the Lead Registrant's dossier.

Comparison of acute toxicity in humans with dose levels required for developmental toxicity

in rodents

The reported lethal doses in humans after single oral uptake are in the range of 300 to 1000 mg/kg bw (IPCS, 1997). By comparison, the LD50 values in animals are typically in the range of 2000 to 17000 mg/kg bw. High doses of methanol are also associated with teratogenicity in rodent developmental studies, with repeated doses causing such adverse effects being above 1000 mg/kg bw/d.

Blood methanol levels in humans would not approach those associated with developmental toxicity of ≥537 mg/L in mice or ≥1840 mg/L in rats without severe and potentially lethal acute toxicity[5]. Furthermore, such levels are associated with formate accumulation and metabolic acidosis in humans. Specifically, formation of formate can exceed its subsequent oxidation in the metabolic pathway in primates, which is particularly important because the toxicity of methanol in humans appears linked to formate[6].

[5] A blood level of 500 mg/L methanol in acutely poisoned patients generally is regarded as requiring hemodialysis.

[6] As discussed in Section 5.1.3 on toxicokinetics of the Lead Registrant's CSR, the methanol dose that saturates the folate pathway in humans is estimated at ≥200 mg/kg bw and toxic blood formate concentrations are reported to be ≥220 mg/L.

In ECHA Guidance on the Application of the CLP Criteria Version 4, 2013, Section 3.1.6.1.1 the example for methanol is given, with the rationale for not classifying for acute toxicity based on the animal data: *"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)"* Similarly for the Classification for STOT-SE in Section 3.8.6.1.1 for methanol the rationale for not classifying based on animal data is the same as above, and instead classification based on human data is given as: *"The classification criteria for category 1 are fulfilled: clear human evidence of a specific target organ toxicity effect which is not covered by acute toxicity."*

The major differences in metabolism between rodents (mice and rats) on the one hand resulting in high circulating methanol levels, and rabbits, primates and humans on the other hand, resulting in high circulating formate levels, means that one cannot have a "strong presumption" that the results of developmental toxicity studies in rodents can be applied directly to humans, as is required under the CLP Regulation for classification to be applied.

CLP classification rules

The proposed classification of methanol for reproductive toxicity does not appear consistent with CLP criteria.

Available occupational epidemiological data have not considered developmental toxicity, with the exception of one study, and poison centre case reports are compromised by multiple exposures and other uncertainties, (NTP, 2003)[7]. Overall there is not an association evident between methanol exposure and developmental toxicity in humans. If methanol is a human teratogen then incidences from poisonings would likely to have been identified by physicians and reported. A proposal for classification would therefore need to be based on animal studies and present the case that *"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"* while taking into consideration whether this may occur with other toxic effects (Table 3.7.1(a) of the CLP Regulation).

[7] There are no relevant epidemiological studies or case reports which describe an increase in the

incidence of malformations in children of mothers exposed to methanol during pregnancy.

The CLP Regulation states that a substance should not be classified when a *"clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans"* (Section 3.7.2.3.2). In cases when mechanistic information only raises doubt about the relevance of the effect on developmental toxicity for humans, the CLP Regulation establishes that classification in category 2 may be more appropriate than category 1: *"…when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate"* (Table 3.7.1(a)).
A decision on classification should be made on the basis of *"an assessment of the total weight of evidence"* (Section 3.7.2.2.1). There are significant data available on both the toxicokinetic differences and mechanism of action which enable a robust conclusion: methanol should not be classified as a selective reproductive toxicant in humans according to CLP rules. When considering the weight of evidence, it is clear that there are significant species differences with regards to the toxicity of methanol, as a result of metabolism (of methanol) and toxicokinetics. Species differences in metabolism and toxicity of methanol are well-established in toxicology, with a majority of research on the subject being conducted in the 1980s. For humans, metabolism of methanol to formic acid requires specific consideration due to acute toxicity and the fact that formic acid is not classified as a reproductive toxicant.
Furthermore, recent mechanistic investigations do not support a relevance of methanol developmental toxicity in rodents to humans, as these indicate that developmental toxicity may be caused by reactive oxygen species from metabolism of high doses of methanol by catalase (MacAllister *et al*., 2011; McCallum *et al*., 2011; Miller & Wells, 2011). A mechanism involving catalase is known to not have a relevance to humans, due to the different metabolism for methanol in humans.
Given the significant acute toxicity of methanol in humans, it is unlikely that methanol has the capacity to interfere with reproduction in humans without other toxicity severely impacting the mother or foetus. This scenario cannot be replicated in rodent studies, due to the difference in metabolism of methanol: in rodents, there is a lack of acidosis and ophthalmologic changes, whereas in humans these toxicological effects are eminent, due to formic acid formation. Methanol oxidation becomes saturated in the rodent model with a Km approx. 10 times lower than for humans at relevant exposures (Perkins *et al*., 1995), whereas the rate of oxidation of formate is approximately 40 times lower in humans (Sweeting *et al*., 2010).

Together, this demonstrates the marked differences between humans and rodents, which are critical when considering that developmental toxicity in rodents is only observed at high blood methanol concentrations (≥537 mg/L in mice and ≥1840 mg/L in rats).

Conclusion

<u>The Methanol REACH Consortium does not agree with the CLH dossier that methanol should be classified with Reproductive Toxicity category 1B:</u> In our opinion, the same type of reasoning that has been used in classifying methanol for acute toxicity and for specific target organ toxicity, but in reverse, should be applied to consideration of the data for developmental toxicity.

The clear data for methanol induced teratogenesis in rodents at high dose levels are not considered to be a good model for human effects. The data are not relevant for classification in humans since primate data and supporting rabbit data have not demonstrated teratogenic effects, and it is not possible to expose primates and humans to

such high dose levels as rodents. It follows that methanol should not be classified for developmental toxicity for human health as was previously agreed by the Classification Committee under the Dangerous Substances Directive.

Further References:

ECHA (2012) REACH registration dossier for sodium formate – disseminated endpoint study records

HC, Health Council of the Netherlands (2006) Evaluation of the effects on reproduction, recommendation for classification. Committee for Compounds toxic to reproduction A Committee of the Health Council of the Netherlands. No. 2006/04OSH, The Hague, June 13, 2006

HC (2010) Health-based recommended occupational exposure limit. Dutch Expert Committee on Occupational Safety a Committee of the Health Council of the Netherlands. No. 2010/01OSH, The Hague, January 21, 2010

HEI, Health Effects Institute (1999) Reproductive and Offspring Developmental Effects Following Maternal Inhalation Exposure to Methanol in Nonhuman Primates. Research Report Number 89. October 1999

International Programme on Chemical Safety (IPCS) (1997). Methanol. Environmental Health Criteria 196. World Health Organisation, Geneva

MacAllister S.L., Choi J., Dedina L., and O'Brien P.J. (2011) Metabolic mechanisms of methanol/formaldehyde in isolated rat hepatocytes: carbonyl-metabolizing enzymes versus oxidative stress. Chem Biol Interact. 191(1-3):308-314

McCallum G.P., Siu M., Ondovcik S.L., Sweeting J.N., and Wells P.G. (2011). Methanol exposure does not lead to accumulation of oxidative DNA damage in bone marrow and spleen of mice, rabbits or primates. Mol Carcinog. 50(3):163-172

Miller, M.J. & Wells, P.G. (2011) Altered methanol embryopathies in embryo culture with mutant catalase-deficient mice and transgenic mice expressing human catalase. Toxicol. Appl. Pharmacol. 252: 55-61

Perkins, R.A, Ward, K.W., and Pollack, G.M. (1995) A pharmacokinetic model of inhaled methanol in humans and comparison to methanol disposition in mice and rats. Environ. Health Perpect. 103(7-8):726-33

NTP, National Toxicology Program (2003) NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Methanol. NIH Publication No. 03-4478, Sept 2003

Siu M.T., Wiley M.J., and Wells P.G. (2013) Methanol teratogenicity in mutant mice with deficient catalase activity and transgenic mice expressing human catalase. Reprod Toxicol. 36: 33-39

Sweeting J.N., Siu M., McCallum G.P., Miller L., and Wells P.G. (2010) Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates. Toxicol Appl Pharmacol. 247(1):28-35

Sweeting J.N., Siu M.T., Wiley M.J., and Wells P.G. (2011) Species- and strain-dependent

teratogenicity of methanol in rabbits and mice. Reprod Toxicol. 31(1):50-58

[ECHA note: End of attachment 1]

Dossier Submitter's Response

Thank you for the comment. Please refer to the response to comment number 2.

RAC's response

Thanks for the information and the detailed comment. In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the RAC agrees that the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2013	Netherlands	RIVM	National Authority	12

Comment received

Reproductive toxicity

Current classification for reproduction: none
Proposal: Repro. 1B (H360D)

The Netherlands agrees with the Italy MSCA in that there is good evidence that the mechanism for the developmental toxicity induced by methanol is relevant to humans and a classification of methanol in Repro. Cat. 1B ('substances which should be regarded as if they cause developmental toxicity in humans') and labeling with H360D ('may cause harm to the unborn child') is warranted.

Dossier Submitter's Response

Thank you for the comment.

RAC's response

Classification as Repr. 1B requires that there is a strong presumption that the effects will occur in humans.
In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	Germany		Individual	13
Comment received				
In order to maintain a proper working classification system in Europe that informs on the scientifically observed hazards with a reasonable relevance to humans it is proposed to not classify methanol for developmental effects. The current classification sufficiently informs humans on methanol toxicity and thus enables a proper risk management. Further information is provided in the attached document. *ECHA's note: The further information was provided in the following attachment:* *PERSONAL OPINION ON THE CLASSIFICATION OF* *METHANOL AS DEVELOPMENTAL TOXICANT [attachment 3]*				
Dossier Submitter's Response				
Thank you for the comment. Please refer to the response to comment number 2.				
RAC's response				
The RAC has noted that in contrast to rodents, humans have an extremely limited capacity of the folate metabolism leading finally to life threatening acidosis and damage of the optic nerve. Based on an overall assessment of the data, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. The RAC therefore argues that methanol should not be classified for developmental toxicity.				

Date	Country	Organisation	Type of Organisation	Comment number
09.12.2013	Germany		MemberState	14
Comment received				
p. 47/48: The DE CA does not support the conclusion for the proposal to classify the substance as Repr. 1B – H360D. Based on the data presented in the dossier, classification as Repr. 2 ("Suspected human reproductive toxicant") appears more appropriate instead. As stated in the dossier, there are substantial species differences in methanol (MeOH) metabolism between rodents and non-rodents. Humans, monkeys and rabbits metabolise MeOH using ADH, rodents using the catalase-peroxidase pathway probably leading to accumulation of different metabolites. MoA and the identification of the proximate toxicant are still under debate and the lines of arguments for in vivo/in vitro comparison are not as clear as presented. The clear developmental effects observed in rodent studies are acknowledged but the relevance for humans, i.e. the "strong presumption that MeOH has the capacity to interfere with reproduction in humans" according to CLP regulation, remains to be clarified. On the other hand, the findings of the available rabbit and monkey studies which according to the dossier better reflect the human situation, may be biologically relevant but lack statistical significance and standardization. For the current proposal, it is considered necessary to describe the studies which serve as basis for the classification proposal in more detail and to further substantiate justification for categorisation. p. 44/p.46: The study by Sweeting et al. (2011) is cited as WoE evidence for the substance acting as a teratogen in rabbits. Contrary to this, the authors of the study emphasized the resistance of the New Zealand white rabbit towards MeOH-induced teratogenicity , though acknowledging that some non-significant developmental effects were observed (which are not observed in rodents). Interestingly, the study also provided evidence that the				

susceptibility of mice to MeOH reproductive toxicity is highly strain-specific.
Similarly, the reliability of the cited monkey studies (Burbacher at al. (1999, 2004) reporting shortened mean gestational period, pregnancy complications and developmental neurobehavioural effects is questioned. The mentioned Dutch report from 2010 (p. 10) explicitly discarded the 1999 study as being inconclusive. A recent toxicological review of Methanol (Noncancer) by EPA (2013) concluded that "there is insufficient evidence to determine if the primate fetus is more or less sensitive than rodents to methanol teratogenesis", though several developmental effects were rated as being biologically significant (not the least, as doses as low as 260 mg/m3 were effective).

p. 10/47: Accordingly, the CLH report's statement that development is severely impacted in rabbits and monkeys has to be regarded with care and need further robust investigation.

p. 45/46: A number of in vitro and mechanistic studies are cited intending to support the hypothesis that MeOH developmental effects observed in rats and mice are not unique to rodents and providing clues on the identity of the proximate toxicant. In particular, the importance of reactive oxygen species (ROS) in bringing about MeOH-induced embryotoxicity has been highlighted, based on whole embryo cultures (WBC) and transgenic studies.
In general, in vitro studies fail to provide reliable information on the toxic activity of metabolites. More specifically, it has been reported very recently, that in contrast to mouse WBC, ROS did not contribute to teratogenic effects in an in vivo mouse model. Moreover, it provided evidence that glutathione (GSH) does not primarily protect from teratogenesis as a ROS scavenger but rather as a co-factor of ADH3 activity. ADH3 is a principal enzyme involved in human MeOH metabolism (blocking GSH results in accumulation of formaldehyde as ADH3 is unable to transform formaldehyde to formic acid). In other words, this study challenges the teratogenic relevance of formic acid and ROS and identifies formaldehyde as the putative proximate teratogen, despite its reactivity and rapid decay (Siu et al., 2013).
Although preliminary, this data shows the current uncertainty of the role of species-specific metabolic events and toxic metabolite, requiring further robust investigation.
Overall, the data presented cast doubt whether classification of the substance as Repr. Cat 1B is appropriate.

Dossier Submitter's Response

We acknowledge that there are several uncertainties in the evaluation of MeOH developmental toxicity; however, in our opinion the weight of the evidence indicate that classification as 1b is more appropriate.
let's consider
- human data: the limited human data can only suggest that high exposure to MeOH during pregnancy may lead to serious foetal and neonatal toxicity. Moreover, the avalable data cannot allow to assess whether acute toxicity would prevent the onset of developmental effects at equal or lower exposure levels.The findings of human studies are too limited to allow a conclusion, therefore the assessment of MeOH developmental toxicity should rely on experimental data.
- animal data: although the strength of the evidence and the quality of studies is not the same for each species, the available studies indicate that MeOH produces severe developmental effects in two rodent (rat, mouse) and two non-rodent (rabbit, Macaca mulatta) species in absence of maternal toxicity; such developmental effects include also teratogenicity in both rodent species as well as in rabbits.
- mode(s) of action: several modes of action appear to be involved in MeOH developmental toxicity, including the generation of reactive oxygen species, the interference with placental functions and the production of formaldehyde by a folate-dependent pathway. There is no

evidence that such mechanisms might be not relevant to humans; actually, interference with placental function has been reported also in MeOH-exposed human placental explants in vivo. Moreover, the ability to metabolize MeOH, as well as the vulnerability to reactive oxygen species may show a considerable variability in humans as a result of genetic and environmental factors; therefore the available data suggeast that some subjects and/or subgroups could be higly susceptible to MeOH developmental toxicity.
Therefore, the currently available data set indicates that classification of MeOH as developmental toxicant Repr. 1B – H360D is both adequately conservative and scientifically justified.

RAC's response

The RAC agrees with many of the points raised in the comment. The toxicological effects in rabbits and primates are not very robust, and clearly not sufficient for classification.
In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
13.12.2013	Italy	IReS	Industry or trade association	15

Comment received

It is well known that in humans methanol produces life-threatening acute poisoning after a single dose of 0.3-1.0 g/kg and that ingestion of 100-200 mL of methanol is fatal to most adults. These amounts are associated with serum levels > 20 mg/dL (an ingestion of 0.25 mL/kg of 100% methanol would theoretically - assuming 100% absorption - result in a toxic methanol concentration) [1, 2]. Antidotal treatment is required at serum levels > 20 mg/dL and severe, life-threatening toxicity requiring intensive care support is observed in patients with serum levels > 50 mg/dL [3].

In this perspective, the data discussed in the CHL report [4] present the following critical issues.

- In experimental studies, at oral doses in the order of several grams/kg methanol no effects on maternal toxicity were reported, while developmental toxicity was observed. The same doses in humans are expected to cause life-threatening acute toxicity.

- In inhalation studies, methanol blood concentration at the end of the exposure was investigated by Rogers and Mole (1997), and it was about 4 mg/mL (400 mg/dL) after 10000 ppm exposure for 7 hours/day. This exposure level has been used in other studies. Once again, the observed developmental effects occurred in animals at methanol internal doses that would be incompatible with life in humans.

- Finally, in in vitro studies embriotoxic effects were observed at dose levels that exceed acute, toxic levels in humans: in vitro concentrations of 2-16 mg/mL would correspond to

200-1600 mg/dL at cellular level, largely greater than the serum toxic level of 50 mg/dL previously mentioned.

Therefore, in our opinion, the observed developmental toxicity in animals can not be considered relevant for humans: the classification as 1B requires a "strong presumption that the substance has the capacity to interfere with reproduction in humans" and this strong presumption is lacking. Effects observed at doses that are lethal in humans do not represent a strong presumption.

However, available experimental data should not be discarded, but according to the actual scientific knowledge they should be regarded as inconclusive in order to define the reprotoxic potential of methanol, and further investigations are needed. In particular, since animal models clearly proved to be unsuitable as they are for studying this endpoint, the assessment of interspecies toxicokinetics differences with in silico (e.g. by PB-PK comparison) and/or in vitro approaches would represent a rationale way to properly re-evaluate the relevance for humans of available data.

References

[1] Poisindex Managements. Methanol. In: Klasco RK (Ed): POISINDEX® System. Truven Health Analytics, Greenwood Village, Colorado.

[2] Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 5th ed, Chemical Toxicology Institute, Foster City, CA, 2000

[3] The American Academy of Clinical Toxicology Ad Hoc Committee on the Treatment Guidelines for Methanol Poisoning: Donald G. Barceloux, G. Randall Bond, Edward P. Krenzelok, Hannah Cooper, and J. Allister Vale. American Academy of Clinical Toxicology Practice Guidelines on the Treatment of Methanol Poisoning. Clinical Toxicology, 40(4), 415–446 (2002).

[4] Istituto Superiore di Sanità (on behalf of the Italy MSCA). CLH report. Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 for the substance Methanol. Version number 3, October 2013.

Dossier Submitter's Response

The Italian CA can agree that more mechanistic data would support the evaluation of MeOH developmental toxicity. Hovever, whereas uncertainties have to be recognised, the current available data set has to be considered relevant for classification.
MeOH consistently induced developmental effects in the absence of maternal toxicity in in vivo studies on rodent and non-rodent laboratory animal species (rats, mice, rabbit and monkey) performed by the most relevant route of exposure (inhalation) in the absence of maternal toxicity.
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. The findings of human studies are too limited to allow a conclusion concerning the developmental toxicity of MeOH. Therefore the assessment of MeOH developmental toxicity should rely on experimental data.
It is acknowledged that MeOH has a considerable acute toxicity in humans; however the avalable data cannot allow to conclude that such acute toxicity would prevent the onset of developmental effects at equal or lower exposure levels.

The mechanisms underlying the developmental effects of MeOH in rodents and in rabbit involve several modes of action, including the generation of reactive oxygen species, the interference with placental functions (observed also in human explants *ex vivo*) and the production of formaldehyde by a folate-dependent pathway. There is no evidence that such mechanisms might be not relevant to humans. Moreover, the ability to metabolize MeOH, as well as the vulnerability to reactive oxygens species may show a considerable variability in humans as a result of genetic and environmental factors; therefore the available data suggeast that some subjects and/or subgroups could be higly susceptible to MeOH developmental toxicity. Based on the above considerations, the classification of MeOH as developmental toxicant cat Repr. 1B – H360D is regarded as both adequately conservative and scientifically justified.
RAC's response
In order to decide whether the clearly positive rodent data leads to a strong presumption that methanol can affect reproduction in humans, the metabolic differences between rodents and humans have to be evaluated. Big differences in metabolism between rodents and humans results in very different toxicity profiles in rodents and humans, and the RAC is therefore of the opinion that the rodent toxicity data cannot be used in isolation for the classification. Based on a comparison of blood methanol concentrations in rodents at developmental toxicity LOAECs and blood methanol concentrations in humans resulting in acute toxicity, the RAC is of the opinion that the high acute toxicity of methanol in humans (via the formate metabolite) will result in acute toxicity before any developmental toxicity can be expressed in humans. Methanol is classified for acute toxicity and STOT SE, and in the opinion of RAC, methanol should not be classified for developmental toxicity.

ATTACHMENTS RECEIVED: 3

1. *Consultation C&L Methanol (Filename: Final comments_CLH Dossier_Methanol_10-12-2013.pdf),* submitted by REACH Methanol Consortium on 11/12/2013. Refer to comment no. 8 and 11.

2. *Methanol Institute Comments on the Proposed Classification of Methanol as a Reproductive Toxicant Under the CLP Regulation (Filename: MI_Comments_on_Proposed_Classification_Methanol_Under_CPL_Regulation-FINAL.pdf),* submitted by Methanol Institute on 12/12/2013. Refer to comment no. 7.

3. *PERSONAL OPINION ON THE CLASSIFICATION OF METHANOL AS DEVELOPMENTAL TOXICANT (Filename: Statement on classification as reprotoxic Cat 1B.pdf),* submitted by individual on 13/12/2013. Refer to comment no. 2 and 13.