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

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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.

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."*

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

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.

<u>Interspecies differences of methanol toxicity and developmental toxicity of methanol</u>

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.

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.

[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.

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.

<u>Comparison of acute toxicity in humans with dose levels required for developmental toxicity in rodents</u>

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].

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

[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.

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).

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

[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.

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

The Methanol REACH Consortium does not agree with the CLH dossier that methanol should be classified with Reproductive Toxicity category 1B: 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:

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