Methanol Institute

Avenue Jules Bordet, 142, 1140 Brussels, Belgium, Tel: +32 276 116 59

Methanol Institute Comments on the Proposed Classification of Methanol as a Reproductive Toxicant Under the CLP Regulation

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 carbon dioxide. 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 carbon dioxide 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 ethanol-metabolism 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.