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# Discussion paper / probit function methanol

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| Date: | 20 May, 2009 |
| **Comments before:** | **1 July, 2009** |
| Document id: | 20090520-probit-methanol |
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# Discussion: Deriving a probit function for methanol

The derivation of a human probit function for methanol requires special attention. The metabolism of methanol in humans and non-human primates is both quantitatively and qualitatively different compared to that in rodents. It appears that primates are more sensitive to methanol exposure than rodents, caused by a faster detoxification process in rodents. The AEGL committee, ERPG committee and the Dutch Health Council state that rodents are not an appropriate animal model for humans in the case of methanol toxicity. For this reason, human and/or non-human primate data should be used for deriving guideline levels or in this case deriving a probit function for lethality for methanol. Three studies in monkeys have been identified, where the animals were once or repeatedly exposed to methanol. Unfortunately, none of the studies has been assessed fit for probit function derivation. Finally, two options are provided as alternative approaches to derive a probit function.

**Metabolism of primates versus rodents**

The metabolism of methanol in primates and rodents are schematically shown in the figure below.

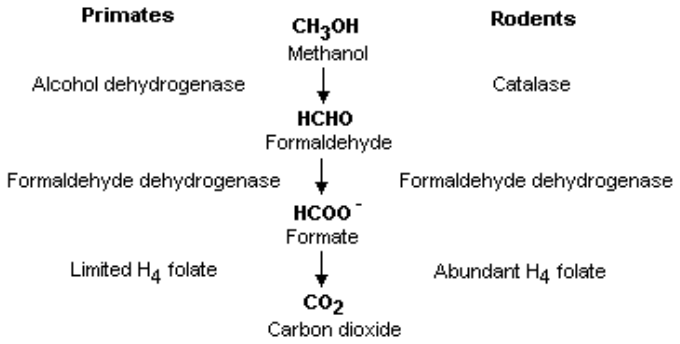


Figure 1 Scheme for the metabolism of methanol. Major enzymes for primates (left) and rodents (right) are noted. Species differences in methanol toxicity are due primarily due to the metabolic conversion of formate to carbon dioxide, which is rapid in rodents but slow in primates.

In both primates and rodents methanol is metabolized to formaldehyde and further to formate, which is then either excreted or further oxidized to carbon dioxide. In rodents, the catalase enzyme metabolizes methanol to formaldehyde in contrast to the alcohol dehydrogenase enzyme, which is active in primates. Another major difference in the metabolism is the rate of detoxification of the metabolite formate. In rodents, this process is fast due to the abundant $H_4$ folate enzyme. In primates, however, due to a limited amount of this enzyme formate is allowed to accumulate, especially at high concentrations. The accumulation of formate will consequently lead to metabolic acidosis; a toxic effect rarely observed in rodents.

**Critical effects and cause of death**

Critical effects are effects on the central nervous system (CNS) and metabolic acidosis. The former effect starts rapidly after exposure and is likely caused by methanol itself at first and later on also by metabolites. This effect is observed in rodents and primates alike. Metabolic acidosis is the result of formate formation and often ‘delayed’. It is, however, not known whether saturation in the oxidation of formate occurs at concentration levels below levels at which methanol causes a depression of the CNS. Therefore, it is unknown which effect is most critical.

Relevant for the probit function discussion is the cause of death after methanol exposure. It is very likely that both effects are present at high exposures. Up until now, it is unknown whether CNS effects or metabolic acidosis or the combination thereof are the cause of death.

Since metabolic acidosis cannot be ruled out as (partial) cause of death and rodents do not display this type of effect after methanol exposure, rodent data should not be considered as model for human lethality.

**Non-human primates data concerning lethality**

McCord (1931) studied the toxicity of methanol following skin absorption and inhalation in young rhesus monkeys (from the wild), rabbits (four different breeds) and white rats. The data on rhesus monkeys only will be described. The rhesus monkeys were exposed to either synthetic, pure natural, 95% natural or crude natural methanol. Methanol was introduced to the animals by vapour due to heating with a light bulb. Exposure to (target) concentrations of 1,330 (4 animals), 6,650 (1), 13,300 (2), 26,600 (1), 53,200 (3) mg/m$^3$ was maintained by using a dripping apparatus during 1 to 18 hours. The study description lacks detail on exact exposure conditions per animal or group. The author states that exposure to 53,200 mg/m$^3$ methanol for 1 to 4 hours lead to death (delayed or in case of 4 hours prompt death). The author further stated that one monkey may long survive the action of 6,650 mg/m$^3$, while another is promptly killed by 1,330 mg/m$^3$. Unfortunately, the author did not describe the fate of the other animals during the study and observation period (of which the length was unknown).

In a subchronic inhalation study, Andrews et al., 1987, exposed male and female cynomolgus monkeys (3/sex/group) to analytical concentrations of 692, 2,633, and 6,663 mg/m$^3$ methanol for 6 h/d, 5 d/week for 4 weeks. All animals tested, including rats (5/sex/group), survived the duration of the study.

The New Energy Development Organisation (NEDO, 1987, original report not available, based on summary reports in AEGL document) performed acute and chronic toxicological studies in monkeys, rats and mice. In the acute study in monkeys (macaques) were exposed to 3990 (4), 6650 (3), 9310 (1) or 13300 (2) mg/m$^3$ for 21h/d for several days, up to 20 days or until death. All animals in the lowest concentration group survived, all animals in the higher concentration groups (at 6650 mg/m$^3$ or higher) died or had to be killed after at least three days.
In the chronic experiment, monkeys (macaques) were exposed to 1330 (5), 2660 (3), or 3990 (4) mg/m$^3$ for 21 h/day for 7 months, and sacrificed after recovery periods of 0, 1, 6,or 10 months for pathological analysis. None of the animals died during the experiment.
In another experiment of this series: eight female monkeys (macaques) per group were exposed to 13.1, 131 or 1310 mg/m$^3$ for 21 h/day for up to 29 months. None of the animals died during the experiment.

Lethality in the rat after methanol exposure show $LC_{50}$ values of 193,000 (1hr), 85,000 – 130,000 (4hr) and 89,000 (6hr) mg/m$^3$ (cited in AEGL document).

*Based on the data in primates it is not possible to derive a reliable probit function for methanol*.

**Other options**

There are two alternatives for deriving a probit function. The first is to base the probit function on internal blood methanol levels in a similar way as described in the AEGL document. Case studies describing methanol ingestion that resulted in deaths are numerous. These case studies have been well documented including time period after ingestion, hospitalisation period, time of death and blood methanol concentration. The AEGL utilised this information to derive the point of departure for the AEGL-3 level. Subsequently, the blood methanol concentration associated with the threshold of lethality in humans was used in a pharmacokinetic model to calculate the external concentration required to obtain that blood methanol concentration. However, this approach cannot be applied to probit function derivation since data is lacking on the concentration-response relationship and do not allow a

reliable estimation of the human $LC_{50}$ value in the normal procedure. In order to overcome this lack of information the threshold of lethality could be assumed to represent the $LC_{0.1}$ and an n-value set at 2 as default. Conditional there is sufficient confidence in the pharmacokinetic model and point of departure.

The second alternative is to use the rat data in spite of the metabolic differences. In this case it is suggested to determine an additional correction factor on top of the other extrapolation factors to derive the human $LC_{50}$ value. In this option, the default of n = 2 should be used, since any n-value in rodents is not predictive for humans.

Logically a third option exists, that is not to derive a probit function for methanol.