20220608-Methanol
-INHOUDELIJK VASTGESTELD

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Probit function technical support document

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substance name	CAS number
Methanol	**67-56-1**

This document describes the derivation of a probit function for application in a quantitative risk analysis (QRA). The probit function has been derived according to the methodology described in RIVM report 2015-0102.

This document has been checked for completeness by the Netherlands' National Institute of Public Health and the Environment (RIVM). The contents of this document, including the probit function, has been approved by the Dutch Expert Panel on Probit Functions on scientific grounds. External parties have had the opportunity to comment on the derivation of the proposed probit function. The status of this document has now been raised to "inhoudelijk vastgesteld" (approved content).

Detailed information on the procedures for the derivation, evaluation and formalization of probit functions is available at
http://www.rivm.nl/en/Topics/P/Probit_functions

Technical support document Methanol

1. Substance identification

CAS-number:	67-56-1
IUPAC name:	Methanol
Synonyms:	Methyl alcohol
Molecular formula:	CH_3OH
Molecular weight:	32 g/mol
Physical state:	liquid (at 20°C and 101.3 kPa)
Boiling point:	65°C (at 101.3 kPa)
Vapour pressure:	12.8 kPa (at 20°C)
Saturated vapor conc:	128.000 ppm = 171 g/m^3 (at 20°C)
Conversion factor:	1 mg/m^3 = 0.75 ppm (at 20°C and 101.3 kPa)
	1 ppm = 1.33 mg/m^3 (at 20°C and 101.3 kPa)
Labelling:	H301-311-331-370

2. Mechanism of action and toxicological effects following acute exposure

Special considerations:

The metabolism of methanol is different in humans and non-human primates compared to rodents. Humans, non-human primates and rodents eliminate methanol by metabolism to formaldehyde and further to formate, which is then either excreted or further oxidized to carbon dioxide. However, the detoxification process, especially that of formate, is faster in rodents than in human and non-human primates. This leads to accumulation of formate during high methanol exposure, resulting in metabolic acidosis. Metabolic acidosis is considered to be the lethal effect in human and non-human primates, whereas in rodents CNS effects are most likely the lethal effect. For this reason, the ERPG committee considers non-human primates a more appropriate model for humans than rodents in case of methanol toxicity. The current interim version[1] shows that the AEGL-3 is based on methanol levels in human blood after an accidental death (from case studies) rather than the use of rodent and non-human primate data. Based on the differences in metabolism of methanol rodent data will not be considered as point of departure for human probit function derivation.

Acute effects:

The main target organs and tissues for inhalation exposure to methanol are the central nervous system (CNS) and ocular tissues. The health endpoints are CNS depression (similar to that seen after ethanol exposure, but milder and transient) and ocular toxicity. Intoxication follows three stages. At first CNS depression occurs, which is primarily due to methanol itself. Secondly a latency period of 6 to 30 hours occurs after which, thirdly clinical health effects develop from formate formation. These effects are CNS lesions resulting in dizziness, nausea and headaches, impaired vision as a consequence of formate-induced retinal damage, and metabolic acidosis. Lethality results from the depletion of the bicarbonate buffer from formate accumulation leading to metabolic acidosis resulting eventually in death with acute cerebral oedema.

Acute exposure may also result in reproductive and developmental effects, which was observed in mice, rats and primates, but no evidence has been found in humans up until now.

Long-term effects: Ocular effects after acute methanol exposure may result in permanent bilateral blindness. Chronic exposure to methanol produces the same effects as after acute exposure. In addition, fatty degeneration and necrosis of the liver and degenerative vacuolisation of the kidney were observed in non-human primates.

[1] The US EPA AEGL program was discontinued at the time when the technical document on methanol was still in the interim phase.

3. Human toxicity data

No reliable and informative studies with details about human inhalation exposure as well as lethality have been identified and described. However, case studies describing methanol ingestion that resulted in death are numerous. These case studies have been documented relatively well, including the time period of blood sampling after ingestion, hospitalisation period, time of death and blood methanol concentrations. Many case studies involve concomitant ingestion of ethanol, which influences (reduces) the toxicity of methanol since alcohol dehydrogenase is limitedly available due to a higher affinity with ethanol. Therefore cases, in which ethanol was also found in blood, were not described nor taken up in further calculations by the AEGL committee or in this document (Appendix 2). The number of cases left is therefore relatively small, i.e. n = 25 (1 case was excluded because of traces of ethanol in the blood leaving 25 cases), and described in 5 case reports by Naraqi et al. 1979; Erlanson et al. 1965; Gonda et al. 1978; Bennet et al. 1953, and Meyer et al. 2000. The age of the subjects ranged from 15 to 65 years, of which in total 6 subjects were female. Eleven subjects eventually died from methanol poisoning, despite of possible medical interventions. The case studies reported the blood methanol concentrations of the patients at a certain time after ingestion (ranging from 4 to 100 hrs post ingestion) and whether the patients survived or not. Measured blood methanol concentrations ranged from 275 mg/L at 52 hrs to 5600 mg/L at 12 hrs for the fatal cases and from 30 mg/L at 100 hrs to 5700 mg/L at 4 hrs for the survivors, respectively. The data are presented in Appendix 3, Table A3.3. It should be noted that the cases included party drinkers, alcohol addicts, and deliberate ingestion of methylated spirits, which suggests a subpopulation of predominantly ‘trained’ alcohol consumers. Furthermore, it should be noted that all subjects underwent treatment. Without treatment the mortality rate certainly would have been higher. The Dutch Poison information centre (NVIC) considers a blood methanol concentration of >500 mg/L as a severe intoxication. At this blood methanol concentration haemodialysis must be pursued immediately.

The AEGL committee utilised clinical information published in practical guidelines on the treatment of methanol poisonings from the American Academy on Clinical Toxicity (AACT) to derive the point of departure for the AEGL-3 level. The AACT uses a threshold level of 500 mg/L methanol in blood independent of time after ingestion, above which immediate treatment is pursued since without treatment this concentration will likely result in death. Gonda et al. (1978) state a minimal lethal dose of 30 mL methanol by ingestion. This value is based on experience without any underlying calculations.
Measured blood methanol concentration is considered by the AACT to be a good proxy for lethality, although likely the lethal effect is metabolic acidosis due to formate formation. Peak concentrations below 200 mg/L usually are associated with asymptomatic individuals (in the absence of co-ingestion of ethanol and a disturbed acid-base balance). The AEGL committee took the 500 mg/L threshold level set by the AACT and applied an intraspecies factor of 3 to take account for more sensitive subjects as clinical experience is based predominantly on adult men and data on females, children, elderly, and subjects with less than optimal folate levels are lacking. A lethality threshold of 167 mg/L methanol in blood was derived. Subsequently, the blood methanol concentration, as associated with the threshold of lethality in humans, was used in a kinetic model by Perkins (1995) to calculate the external concentration required to obtain that blood methanol concentration. The derived AEGL-3 values are given in section 6.

4. Animal acute toxicity data

During the literature search the following technical support documents and databases were consulted:

1. AEGL interim TSD (2005), ERPG (1994) document and reference database for methanol, covering references before and including 1995[2].
2. An additional search covering publications from 1980 onwards was performed in HSDB, MEDline/PubMed, Toxcenter, IUCLID, ECHA, RTECS, IRIS and ToxNet with the following search terms:
   - Substance name and synonyms
   - CAS number
   - lethal*
   - mortal*
   - fatal*
   - LC_{50}, LC
   - probit
3. Unpublished data were sought through networks of toxicological scientists.

Sensory irritation

No studies were identified in which sensory irritation was studied.

5. Probit functions from individual studies

Rodent data are not considered informative for human lethal response to methanol exposure. Therefore, only the available acute lethality data on methanol of non-human primates are considered. However, the available information on the non-human primate data is either poorly described (McCord 1931) or is based on a repeated dose study. Hence an illustrative figure for acute toxicity cannot be given for non-human primates. What can be distilled from the data is that lethality in the non-human primates is observed at 1330 mg/m^3 after an unknown exposure period, and that a concentration up to 6663 mg/m^3 for 6 hrs/day, 5d/week for 4 weeks was without lethality (Andrews et al., 1987). Almost a similar concentration for 21 hrs/day resulted in 100% lethality (NEDO 1987), where the animals died on day 5 and 14, but no mortality at 3990 mg/m^3 under the same exposure conditions, indicating a steep dose-response. The available data in non-human primates (the abovementioned studies) are described in Appendix 1. For comparison, the two reported 4-hr LC_{50} values for rats were 64,000 and 97,900 ppm (85,120 and 130,207 mg/m^3, respectively) (AEGL, 2005).

Appendix 3 contains an overview of human oral intoxication data and back-calculated inhalation concentrations corresponding to the intoxication data. These calculations are based on the Perkins (1995) model implemented in the software program R. The Perkins model includes variability of certain parameters, therefore, the inhalation concentrations are presented with the 5th, 50th and 95th percentiles.

6. Derivation of the human probit function

The available data do not allow deriving a human probit function following the methodology set out in RIVM report 2015-0102. Rodent data are, as abovementioned, not informative for human lethal response to methanol and there are no A or B quality studies with non-human primates available. In an attempt to derive a human probit function based on available data, two alternative approaches have been explored. The first approach is to base the human probit function on the available human oral intoxication data/clinical data in a similar way as done in the AEGL interim document on methanol. The second approach is based on the non-lethal concentration in non-human primates reported in the Andrews et al. 1987 study.

[2] Usually a safe assumption.

Approach 1: human oral intoxication data/clinical data

The use of data from case studies was evaluated, in which oral intoxications with methanol in humans were reported in combination with data from clinicians (see section 3), to derive a human probit function. After evaluating the case studies, it was concluded that case study data cannot be used directly to derive a point of departure, because subjects have been treated to overcome the methanol intoxication. This introduces an unknown effect in the response, which makes the data as such unsuitable for deriving a human probit function, because a reliable lethal dose could not be derived. Therefore, it was decided to follow the approach taken as described in AEGL (2005; see also section 3). Intrinsically, this approach contains some conservatism for the following reasons:

- It describes the methanol blood level upon which immediate action is needed, since without treatment this concentration will likely result in death. This concentration is probably set on the safe side.
- The build-up to the set blood concentration 'treatment' level is different as the oral intoxication is a bolus administration and reaches the maximum blood concentration level in 30 minutes after which blood methanol levels decrease. The build-up after inhalation, in this approach, shows a continuous increase of the blood methanol concentration up until the set 'treatment' level is reached.
- The basic assumption made was to assume that the threshold level of 500 mg/L blood methanol, will result in 50% mortality if subjects are not treated, following the AEGL approach. This is an assumption unsupported by experimental data. Further it disregards the element of time of intoxication. A subject with 500 mg/L methanol in blood after 30 hrs must have had a much higher bolus administration than someone with the same blood level after 10 hrs. However, the approach assumes a similar outcome.

As said, the 500 mg/L blood methanol concentration was used as point of departure and set at 50% mortality. To account for intraspecies differences as clinical data are predominantly based on adult men, a factor of 3 was applied to obtain the 'LD_{01}' of 167 mg/L. Similarly, the value of 167 mg/L was considered the threshold of lethality by the AEGL committee. A blood methanol concentration of 1000 mg/L was associated with an almost certain fatal outcome and thus set at 99% mortality (see Appendix 2). With these assumptions, a kinetic model by Perkins (1995) was used to derive external air concentrations required to result in the predefined blood methanol concentration in humans at the specified exposure durations. The resulting air concentrations (taking the P05 estimates from Table A3.2) were subsequently analysed in DoseResp (Ten Berge, 2016) to provide a human probit function. This choice for taking the P05 would possibly also cover for variability between experienced and non-experienced alcohol consumers, i.e. less trained subjects will likely have higher blood methanol concentrations.

$$Pr = -33.9 + 2.52 \times \ln (C^{1.12} \times t) \text{ with C in mg/m}^3 \text{ and t in min.}$$

The derived human probit function has a scientifically weak basis. In a qualitative sense, there is a strong scientific basis underlying the experience of the clinicians and PBPK model, however in a quantitative sense the scientific basis is weak. The point of departures are not based on empirical evidence, but are based on experience of clinicians of the AACT and the assumption of associated mortality ratios. The influence of medical treatment and the bolus administration of methanol at t = 0 are the cause of the uncertainties of the case studies and thus cannot be used due to bias. Appendix 2 discusses the outcomes if the assumed mortality rates corresponding the 167, 500 and 1000 mg/L blood methanol concentrations would be set to 5, 50 and 95%. It shows that the differences are quite small and would result in the same n-value. Nevertheless, the choices for the blood methanol concentrations resulting in either percentage mortality will have large influence on the derived probit function.

The human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 8368 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 10953 mg/m^3.

Approach 2: non-human primate data as point of departure

The experimental data on methanol obtained in non-human primate data can be considered relevant for humans. None of the non-human primate data on acute inhalation toxicity meet the quality standards specifically for probit function derivation. The data cannot provide a reliable LC_{50} value or n-value as points of departure and therefore are considered as C-study quality. The repeated dose study by Andrews et al. 1987 is a well-performed study in non-human primates that did not result in mortality after concentrations up to 6663 mg/m^3 for 6 hrs/day, 5d/week for 4 weeks. The 6 hr exposure to 6663 mg/m^3 can conservatively be set as a lower limit estimate for a 6hr-LC_{01} point of departure, because of the repeated dosing that was applied in the Andrews et al. study. The study by NEDO seems to be support the 6hr-LC_{01} point of departure as repeated exposure to 3990 mg/m^3 for 21 hrs/day for 20 days did not result in death, whereas exposure to 6650 mg/m^3 resulted in 100% mortality after the 14th day. Since no experimental data is available to derive the n-value is the default value of n = 2 is used. The default factor for interspecies extrapolation is set to one, as non-human primates have a similar metabolism of methanol compared to humans. The assessment factor for database adequacy was set to 1 as the overall database on methanol is considered adequate regarding the availability of toxicokinetic data in rodents and primates, the availability of non-human primate data, and because the LC_{01} is a lower limit estimate considered the overall database. The PoDs for human probit function derivation are set at the 6hr-LC_{01} 6663 mg/m^3 with n = 2. This provides:

$$Pr = -20.83 + 1 \times \ln (C^2 \times t) \text{ with C in } mg/m^3 \text{ and t in min.}$$

Although the experimental study with the cynomolgus monkeys (Andrews et al. 1987) was not an acute exposure study and did not show mortality, it was well performed providing a quantitatively acceptable basis. The probit function can be considered as being a conservative lower limit as no mortality was observed after 4 weeks repeated exposure.

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 11,191 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 16,364 mg/m^3.

Overall discussion

Both approaches have their merits and uncertainties. The case studies and clinical data consider human data; the target species, but stem from oral intoxications where medical interventions and uncertainties in the original exposure dose bias the outcome. Moreover it is likely that the clinical data are precautionary. The uncertainties are large in the sense that the influence of time and human variability in methanol metabolism can have had a major influence on the outcome of survival. In addition, a route-to-route extrapolation was required using a PBPK-model by Perkins, which shows the differences in build-up of blood methanol concentrations over time between oral and inhalation exposure. Nevertheless, the assumptions were set in such way that the selected PoDs are conservative. The question remains how conservative. The non-human primate data on the other hand do not show mortality. The data are derived from a well-performed study with a relevant route of exposure in a relevant species. The repeated exposure provides confidence that the PoD is a non-lethal exposure. To acquire a PoD a mortality percentage needed to be assigned to allow a probit function derivation. The approach with the non-human primate data is also conservative as this mortality percentage was set at 1%.

Preference is given to the non-human primate data as primary source for probit function derivation since the relevant route of exposure was used, in a relevant animal species, where the focus lies on detection of (acute) toxic effects rather than on prevention of health effects by medical intervention. Although the resulting human probit function with approach 2 leads to higher lethality values than under approach 1, the fact that it is based on a study with repeated dosing still provides sufficient confidence that the human probit function does not underestimate methanol lethal toxicity in humans.

In conclusion, the PoDs for human probit function derivation are based on the data from non-human primates and set at a 6hr-LC_{01} 6663 mg/m^3 with n = 2. This provides:

$$Pr = -20.83 + 1 \times \ln(C^2 \times t) \text{ with C in mg/m}^3 \text{ and t in min.}$$

The calculated human 60 min $LC_{0.1}$ (Pr = 1.91) calculated with this probit equation is 11,191 mg/m^3 and the calculated human 60 min LC_1 (Pr = 2.67) is 16,364 mg/m^3.

Table 1 *LC-values calculated with the derived probit function compared with existing acute inhalation exposure guidelines.*

Estimated level	30 min (mg/m^3)	60 min (mg/m^3)
0.1% lethality, this probit	15826	11191
1% lethality, this probit	23142	16364
AEGL-3[3] (2005, interim)	18636	9584
ERPG-3[8] (2016)	-	6650
LBW (2018)	28000	15000

Compared with equivalent (inter)national guideline levels as presented in the table above, the lethal levels derived with this probit function are higher than the ERPG value and similar to the 30-min AEGL value and 60-min LBW.

[3] AEGL and ERPG values were converted from ppm to mg/m^3 with the conversion factor calculated in section 1. Therefore, the AEGL and ERPG values in mg/m^3 can deviate slightly from those reported in the AEGL and ERPG TSDs.

Appendix 1 Animal experimental research

Study ID: C studies

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. The animals were exposed in 4 m^3 exposure chambers constructed of glas and stainless steel with conical tops and bottoms. An average airflow of 2000 l/min was used to provide 1 air change every 3 minutes. The T99 equilibrium time was calculated to be 13.8 min according to the study description. The monkeys were placed on one side of the exposure chamber in meshed cages during testing; rats were placed on the other side. The test atmosphere was generated by ambient flash evaporation. Liquid methanol was introduced in the chamber by a metered flow into an atomizer. The fine aerosols were allowed to evaporate in the air inlet and methanol was introduced as vapour. Test atmosphere was analysed using Wilkes MIRAN infrared analyser. Nominal and analytical concentrations were within limits of expected experimental error (±10%). All animals tested, including rats (5/sex/group), survived the duration of the study.

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. For reasons stated under section 2 (special considerations) only the data on rhesus monkeys 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 established by heating the substance 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).

NEDO (1987) exposed monkeys (Macaca fascicularis) (number of animals and concentration in mg/m^3 given between brackets) to 3000 (4; 3990), 5000 (3; 6650), 7000 (1; 9310) or 10000 (2; 13300) ppm methanol for 21 hours/day for different exposure periods; the control group comprised 6 animals. Continuous monitoring of the exposure concentration revealed mean concentrations of 3053, 5071 and 5018, 7079 and 10441 ppm, respectively. One animal exposed at 10000 ppm showed lethargy and after the third exposure (i.e. the third day) was comatose and died. Another animal exposed to 6000-10000 ppm (duration for different exposure concentrations not clearly stated) died after 6 days. The one animal exposed to 7000 ppm had to be killed after 6 days. Of three animals exposed to 5000 ppm, two died on the 5th day and the third on the 14th day. No lethality was observed in 4 animals exposed at 3000 ppm for 20 days. NEDO (1987) performed chronic toxicological studies in monkeys, rats and mice. Eight female monkeys (macaques) per group were exposed to 13.1, 131 and 1310 mg/m^3 for 21 h/day for up to 29 months. None of the animals died during the experiment.

Appendix 2 Dose response modelling of human data

In literature, a number of cases with methanol intoxication have been described. Many cases involve concomitant ingestion of ethanol, which influences (reduces) the toxicity of methanol because with co-exposure to ethanol alcohol dehydrogenase is limitedly available. The studies with combined exposure were therefore not described. The number of case studies suitable for further calculations is therefore relatively small, n = 25 (one case was excluded), and described in 5 case reports. The ages of the subjects ranged from 15 to 65 years old, of which in total 6 subjects were female. Eleven subjects eventually died from methanol poisoning, despite of possible medical interventions.

It is likely that the cases describe 'trained' alcohol consumers, people that might have adapted to alcohol consumption. Furthermore, it should be emphasized that the subjects have undergone treatment. It is in some cases unclear if treatment started prior to or after blood was drawn and analysed for methanol, resulting in uncertainty of the data. In any case, the outcome of the survivors is largely dependent on the treatment and thus the lethality of methanol is underestimated based on this data. Due to this treatment bias and a subpopulation of possible relatively insensitive subjects it is unclear what the estimate of the 'LD_{50}' may be for the entire population. An additional difficulty arises when one attempts to compare the outcome of survival rate of a bulk exposure at time of ingestion to a gradual prolonged exposure through inhalation of methanol in air resulting in the same blood concentration at e.g. t=4 hours (the 4-hr LC_{50}). The first showing a decreasing trend where the steepness of the decline is determined by the half-life of methanol (2.8 h^{-1}, based on Perkins, 1995) in the human body, whereas by inhalation an increasing trend of blood methanol values will be observed. For the reasons above, it was concluded that the case studies do not provide a solid base for deriving a human LD_{50} and subsequently a human probit function for methanol.

As point of departure for the AEGL-3 values, the AEGL committee used the blood methanol concentration of 500 mg/L, which is considered to be a severe intoxication that needs immediate treatment or otherwise lethality may occur. This 'action level' was based on experience of clinicians of the AACT (see section 3). It should be noted that the clinicians set the 'action level' in the blood, regardless of the time passed after ingestion. Apparently, the blood methanol concentration is a good predictor of the severity of intoxication at time of hospitalization, which is generally relatively late due to the latent effects. It is noted that the 'action level' is more conservative (or favourable) for subjects which are presented early to the clinicians and vice versa. The 'action level' is more or less based on the principle that a critical blood concentration is exceeded and therefore it is considered to be useful for human probit function derivation. For this derivation it was further assumed that the 500 mg/L would result in 50% mortality if subjects were not to be treated. It is acknowledged that there is no direct scientific evidence for this assumption.

The AEGL committee derived what they considered to be the threshold level for lethality of 167 mg/L blood methanol for the entire population. A blood methanol concentration of 1000 mg/L was associated with an almost certain fatal outcome. It was decided to adopt the same approach as the AEGL committee. The 167 mg/L blood methanol concentration was set at 1% mortality and the 1000 mg/L concentration at 99% for modelling purposes.

Using the Perkins (1995) model (see Appendix 3), methanol air concentrations were derived for preset exposure durations (AEGL durations) that would result in either 167 mg/L, 500 mg/L or 1000 mg/L methanol in blood. The Perkins model was used, because it describes the kinetics of methanol in the human body and more specifically in blood. As mentioned above, blood methanol concentrations are considered a good

predictor for lethality. The results of the modelling exercise are shown in Appendix 3, Table A3.2. showing the P05, p50 and p95 variability. Then, the P05 values (lower limit of variation in the population) of the methanol air concentrations were taken forward in probit analysis using DoseResp (Ten Berge, 2016) with their associated durations and mortality rations. Since no actual mortality ratios exist for humans, assumed ratios were inserted representing 1%, 50%, and 99% mortality as 1/100, 50/100 and 99/100, respectively, for the air concentrations corresponding to 167 mg/L, 500 mg/L or 1000 mg/L methanol in blood. Such relatively high group sizes will generate accurate results, meaning small confidence intervals, which in fact are artefacts (of the fictive input for mortality ratios) and the confidence intervals are therefore not given. To study the sensitivity of the input, a second analysis was performed using fictive ratios representing 5%, 50%, and 95% mortality as 5/100, 50/100 and 95/100, respectively, for 167 mg/L, 500 mg/L or 1000 mg/L methanol in blood. The data show no difference in n-value, but do show slightly lower LC_{50} values, because of the more conservative input.

$Pr = a + b \times \ln C + c \times \ln t$

with C for concentration in mg/m^3, and t for time in minutes.

Probit function	*Species*	*a*	*b*	*C*	*n-value*
1%, 50%, 99%	*Human*	*-33.9*	*2.81*	*2.52*	*1.12*
5%, 50%, 95%	*Human*	*-21.5*	*1.92*	*1.72*	*1.12*

The resulting LC_{50} values were:

Duration (minutes)	*LC_{50} (mg/m^3) 1%, 50%, 99% as input*	*LC_{50} (mg/m^3) 5%, 50%, 95% as input*
10	130,200	124,400
30	48,600	46,530
60	26,100	25,020

Appendix 3 Results of the Perkins kinetic model

The kinetic model (equation (1)) of Perkins (1995) is implemented in such a way that the methanol air concentration, which is needed to reach a particular predetermined methanol blood concentration, can be estimated.

$$\frac{dC_b}{dt} = \frac{\Phi V_h C_{inh}}{V_d} - \frac{V_{\max}}{V_d}\left(\frac{C_b}{K_m + C_b}\right) \qquad (1)$$

where

C_b	methanol blood concentration, including background [mg/L]
Φ	fraction of inhaled methanol absorbed into systemic circulation [-]
V_h	ventilation rate [L/kg h]
C_{inh}	methanol air concentration [mg/L]
V_d	volume of distribution [L/kg]
V_{max}	Michaelis-Menten constant of enzymatic methanol oxidation [mg/L]
K_m	maximum rate of enzymatic methanol oxidation [mg/L h]

Since the values of the above parameters will vary within the general population they are not implemented as single (deterministic) values, but as distributions (Table 1). In addition the background blood concentration was defined as a distribution as well (Table 1). The air concentration leading to a particular blood concentration after a particular exposure time was estimated 1000 times hereby randomly drawing values from the input distributions (see Figure 1 as an illustration with 50 iterations). The use of distributions as inputs results in a distribution of the methanol air concentration. This distribution is reported by its median, 5th percentile (P05), and 95th percentile (P95) (Table 2). NOTE that the percentiles do not indicate sensitive or non-sensitive subpopulations. The percentiles do indicate **variation** in the general population. Sensitivity depends on metabolic activation (i.e. formation of formate) rate and elimination of methanol by exhalation and maybe other metabolic and dynamic processes which are not considered in the current model.

The results in Table 2, for example the air concentration leading to a blood concentration of 167 mg/L after 1 hour, should be interpreted as follows. Due to variation in the input parameters some (i.e. 5%) individuals in the population reach a blood concentration of 167 mg/L after exposure to ≤8.8 mg methanol/L air for 1 hour. Exposure to at least 11 mg/L is needed to reach the blood concentration of 167 mg/L after 1 hour. And 95% of the population has a blood concentration of 167 mg/L after 1 hour exposure to 13 mg methanol/L air.

Table A3.1. Definition of the input parameters of the model

Parameter	Type of distribution	Distribution parameter values	Illustrative percentiles of the distribution (P05;P50;P95)	Values obtained from
Φ [-]	beta	alpha: 80 beta: 34	0.63; 0.70; 0.77	AEGL
V_h [L/kg h]	lognormal	GM[a]: 16 GSD[b]: 1.1	14; 16; 19	ECHA 2010 [c]
V_d [L/kg]	uniform	minimum: 0.6 maximum: 0.7	-	Perkins & AEGL
V_{max} [mg/L]	lognormal	GM: 115 GSD: 2.1	34; 115; 390	Perkins
K_m [mg/L]	lognormal	GM: 460 GSD: 2.1	136; 460; 1560	Perkins
Background C_b [mg/L]	lognormal	GM: 1 GSD: 2.2	0.27; 1; 3.7	Perkins

[a] geometric mean
[b] geometric standard deviation
[c] light activity respiration volume of all ages (Table R.15-15)
http://echa.europa.eu/documents/10162/17224/information_requirements_r15_en.pdf

Table A3.2. Methanol air concentrations required to reach a particular blood concentration after a particular time.

Time (h)	Blood concentration (mg/L)	Methanol air concentration [a] (mg/L)		
		P05	P50	P95
0.167 (=10 min)	167 (assumed 1% mortality)	48	59	71
0.5		17	20	25
1		8.8	11	13
4		2.8	3.5	4.4
8		1.8	2.3	3.0
0.167	500 (assumed 50% mortality)	140	180 [b]	220 [b]
0.5		49	60	72
1		26	31	38
4		7.4	9.4	12
8		4.5	6.0	8.1
0.167	1000 (assumed 99% mortality)	290 [b]	350 [b]	430 [b]
0.5		98	120	140
1		51	61	76
4		14	18	23
8		7.9	11	15

[a] based on 1000 blood concentration-time curves
[b] values are above the saturated vapour pressure of approximately 170 g/m^3.

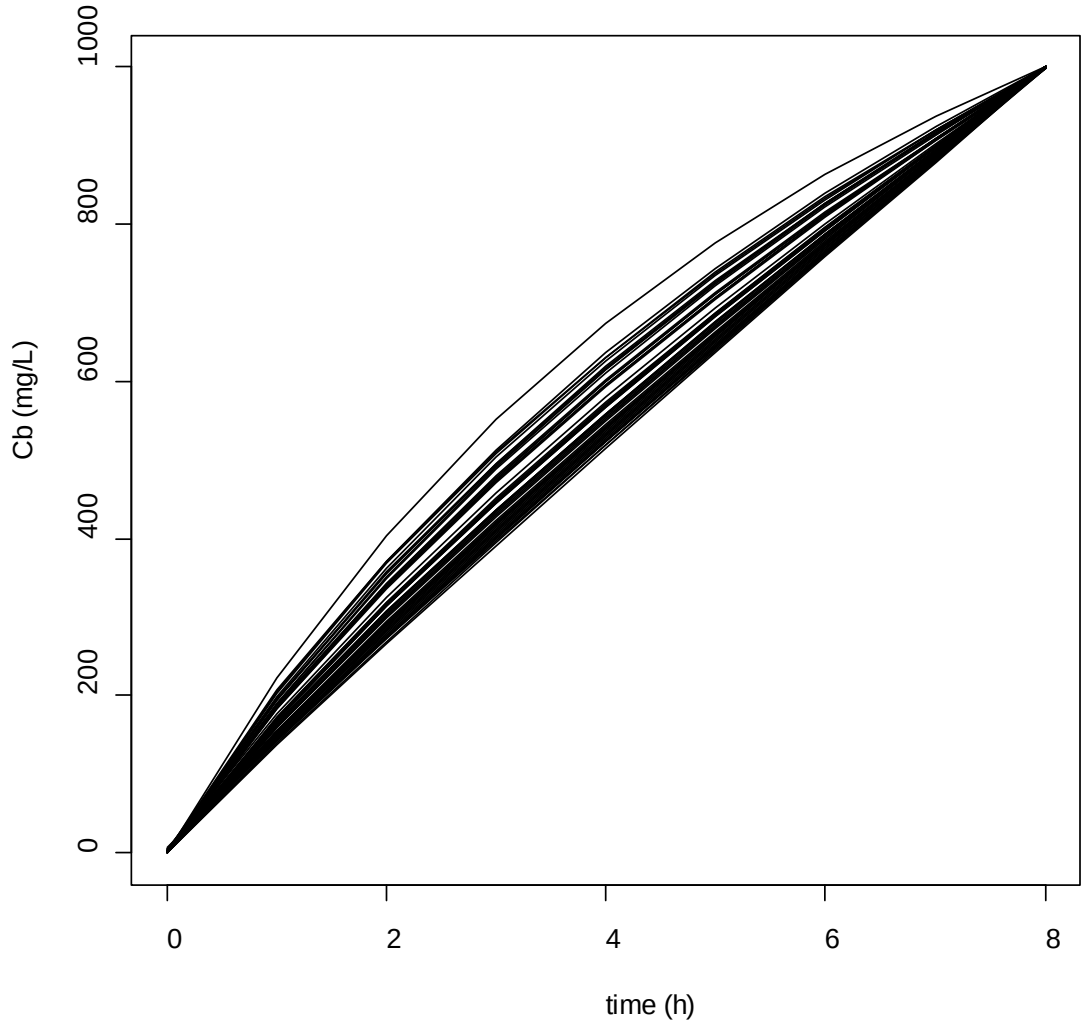


Figure A3.1. Illustration of (50) blood concentration (C_b)-time curves which all result in a blood concentration of 1000 mg/L after 8 hours exposure.

Table A3.3 Back calculated blood methanol concentrations at time of ingestion.

			Cb at t=0 (mg/L)*		
Number.	Measured concentrations Cb (mg/L)	Assumed time of sampling (h)	P05	P50	P95
1 †	730	8	980	1400	2100
2 †	1110	36	2100	4600	11000
3 †	3260	12	3700	4500	6500
4 †	275	52	1800	4700	15000
5 †	277	53	1800	5000	15000
6 †	860	53	2600	5800	16000
7	194	50	1500	4300	12000
8 †	4000	18	4600	5900	9800
9 †	1300	24	2000	3700	7600
10 †	2500	48	4000	7800	21000

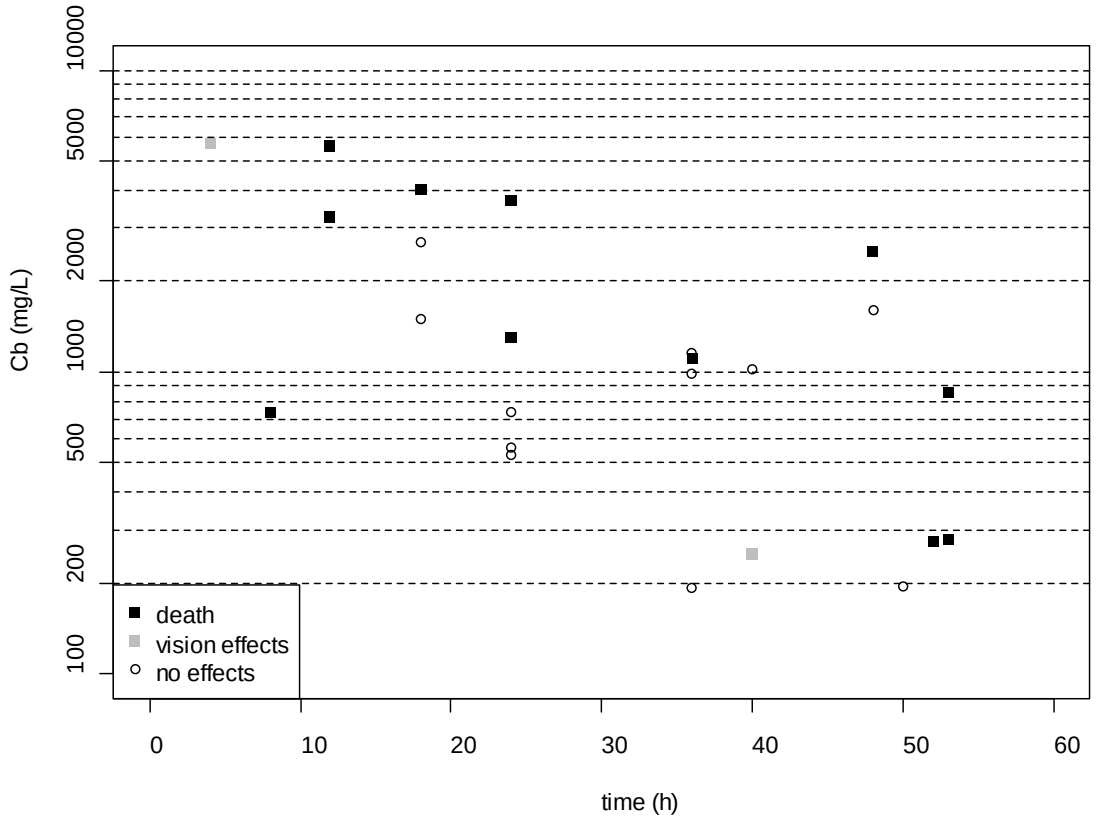
11	1500	18	2100	3300	6600
12	2700	18	3200	4600	8600
13	1600	48	3000	6500	15000
14 †	5600	12	6000	7000	9900
15 †	3700	24	4500	6300	11000
16	5700	4	5800	6200	7000
17	250	40	1300	3500	9700
18	30	100	2500	8700	28000
19	530	24	1200	2500	5700
20	740	24	1500	2800	7700
21	560	24	1300	2700	6400
22	1020	40	2200	5000	13000
23[$]	2050+ethanol	36	3300	5700	13000
24	1150	36	2300	4700	13000
25	990	36	2100	4400	11000
26	192	36	1100	3000	8000

*n=500 iterations.

† subject died of methanol poisoning.

[$] subject not taken forward in calculation due to presence of ethanol in blood.

1



2

3 Figure A3.2 Case studies as reported in AEGL.
4 NOTE: point at t=100 not plotted (number 18), sample with ethanol not plotted
5 (number 23)

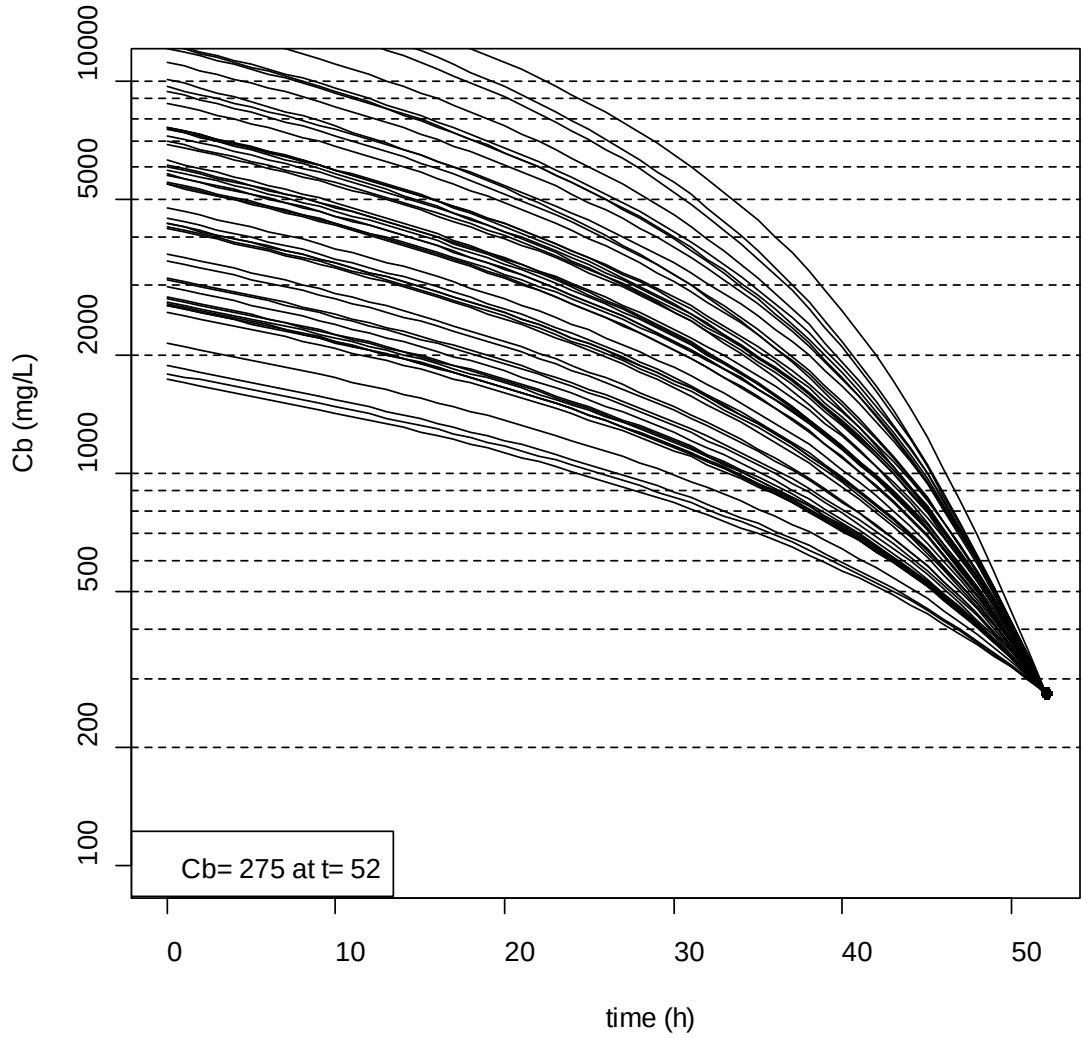


Figure A3.3 Example of back calculation graphically for subject number 4. At 52 hours the blood methanol concentration was 275 mg/L.

Implementation of Perkins model into R

```
meth02<-function(Cinh.start, obs.Cb, time,nloops=1000,it=10)
{
# created on Febr 1, 2012
# by Bas Bokkers
# according to Perkins et al. (1995)
# kinetic model for blood methanol concentrations after inhalation exposure
# Cinh.start: start value [mg/L air] for air conc. leading to particular obs.Cb at time is
end of exposure
# obs.Cb: blood concentration [mg/L blood] to be reached after t=time
# time: duration of exposure (10 min, 0.5,1,4,8 h)
# nloops: number of loops
# it: number of iterations/hour (600 for 10 min, 100 for 0.5h, 10 for rest)

out.loops<-as.data.frame(list(Cinh.opt = rep(0,nloops)))

#Parameters
phi <- rbeta(nloops,80,34)                          # fraction of inhaled
methanol absorbed into systemic circulation
Vh  <- exp(rnorm(nloops,log(16),log(1.1)))          # ventilation rate [L/kg h]
Vd  <- runif(nloops,0.6,0.7)                        # volume of distribution
[L/kg]
Kel <- exp(rnorm(nloops,log(0.25),log(1.1)))        # elimination rate
Km  <- exp(rnorm(nloops,log(460),log(2.1)))         # Michaelis-Menten constant
of enzymatic methanol oxidation [mg/L]
Vmax<- Kel*Km                                       # maximum rate of
enzymatic methanol oxidation [mg/L h]

## Function
metha <- function(t, y, parms) {
with(as.list(c(parms, y)), {
dCb  <- ((phi*Vh*Cinh[t*it+1])/Vd)-(Vmax*Cb)/(Km+Cb)
list(dCb)
})
} # eof

## vector of timesteps
times <- seq(0, time,length=time*it+1)      # time (h)

##Loop to derive Cinh for individual i
for(i in 1:nloops){
print(paste("i=",i))

## Start values
y <- xstart <- c(Cb = exp(rnorm(1,log(1),log(2.2))) )                #startvalues
[mg/L]

parms <- c(phi=phi[i],Vh=Vh[i],Vd=Vd[i],Vmax=Vmax[i],Km=Km[i])

## Optimize Cinh to reach obs.Cb +/- 0.5 at t=time
stop<-F
kk<-0
kkmax<-1000
while(!stop){
kk<-kk+1

```

```
## Dosing
Cinh<-rep(Cinh.start,tstop*it)

## Solving
out <- as.data.frame(lsoda(xstart, times, metha, parms))

if(abs(out$Cb[obs.h*it+1]-obs.Cb)<0.5 || kk > kkmax)
{stop<-T
if(kk>kkmax) print(paste("iteration limit (",kkmax,") reached"))
if(kk==1) print("WARNING kk=1")
}
if(stop==F && out$Cb[obs.h*it+1]-obs.Cb > 0)
Cinh.start<-Cinh.start*0.95
if(stop==F && out$Cb[obs.h*it+1]-obs.Cb < 0)
Cinh.start<-Cinh.start*1.05
} # end of optimization
out.loops$Cinh.opt[i]<-Cinh.start
} # end of loop

##Plotting
if(nloops>1){
hist(out.loops$Cinh.opt,main="",xlab=paste("Cinh (mg/L) at",time,"h"))
GM<-signif(exp(mean(log(out.loops$Cinh.opt))),2)
GSD<-signif(exp(sd(log(out.loops$Cinh.opt))),2)
P95<-signif(quantile(out.loops$Cinh.opt,0.95),2)
P05<-signif(quantile(out.loops$Cinh.opt,0.05),2)
P50<-signif(quantile(out.loops$Cinh.opt,0.5),2)
legend("topright",c(paste("GM=",GM),paste("GSD=",GSD),paste("P05=",P05),paste("
P50=",P50),paste("P95=",P95)))

qqnorm(log10(out.loops$Cinh.opt))
abline(mean(log10(out.loops$Cinh.opt)), sqrt(var(log10(out.loops$Cinh.opt))))
} #end of if
} #eof
```

Appendix 3 Reference list

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