Toxicological Sciences 2006 91(2):576-585

doi:10.1093/toxsci/kfj176

Application of Physiologically Based Pharmacokinetic Modeling in Setting Acute Exposure Guideline Levels for Methylene Chloride

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ABSTRACT

Acute exposure guideline levels (AEGLs) are derived to protect the human population from adverse health effects in case of single exposure due to an accidental release of chemicals into the atmosphere. AEGLs are set at three different levels of increasing toxicity for exposure durations ranging from 10 min to 8 h. In the AEGL setting for methylene chloride, specific additional topics had to be addressed. This included a change of relevant toxicity endpoint within the 10-min to 8-h exposure time range from central nervous system depression caused by the parent compound to formation of carboxyhemoglobin (COHb) via biotransformation to carbon monoxide. Additionally, the biotransformation of methylene chloride includes both a saturable step as well as genetic polymorphism of the glutathione transferase involved. Physiologically based pharmacokinetic modeling was considered to be the appropriate tool to address all these topics in an adequate way. Two available PBPK models were combined and extended with additional algorithms for the estimation of the maximum COHb levels. The model was validated and verified with data obtained from volunteer studies. It was concluded that all the mentioned topics could be adequately accounted for by the PBPK model. The AEGL values as calculated with the model were substantiated by experimental data with volunteers and are concluded to be practically applicable.

INTRODUCTION

Within the framework of the AEGL program, guideline levels are developed for once-in-a-lifetime, short-term exposures to airborne concentrations of acutely toxic, hazardous chemicals by the National Advisory Committee for Acute Exposure Guideline Levels for hazardous substances (NAC/AEGL committee). Acute exposure guideline levels (AEGLs) are meant to provide estimates of exposure concentrations for a range of exposure durations (i.e., 10, 30 min, 1, 4, and 8 h) that are predicted to cause various severities of adverse health effects, i.e., mild (AEGL-1), severe, irreversible, potentially disabling adverse health effects (AEGL-2), or life-threatening effects (AEGL-3) (Krewski *et al.*, 2004).

Data expressing the quantitative relationship between concentration, time, and specific toxicity endpoints are seldom available. Because the issue is an adequate prediction of each of the three AEGL levels rather than aiming at protective but conservative estimates, PBPK modeling is found to be a suitable tool (Bruckner *et al.*, 2004). PBPK models have successfully been applied for compounds like toluene, styrene, trichloroethylene, and xylenes. The present report shows the use of physiologically based pharmacokinetic/pharmacodynamic modeling in setting AEGL values for methylene chloride (dichloromethane, DCM).

The biotransformation of DCM has been well described (Fig. 1). Briefly, two pathways are involved in the biotransformation of DCM, a saturable cytochrome P450–dependent pathway and a glutathione transferase (GST)–dependent pathway. The GST pathway only becomes of importance at relatively high exposure concentrations at which the P450 pathway is saturated. Oxidation by the P450 pathway (probably P450 2E1) via formyl chloride finally leads to the formation of carbon monoxide (CO) and subsequently gives rise to the formation of carboxyhemoglobin (COHb). Formyl chloride can also be conjugated with glutathione (GSH).

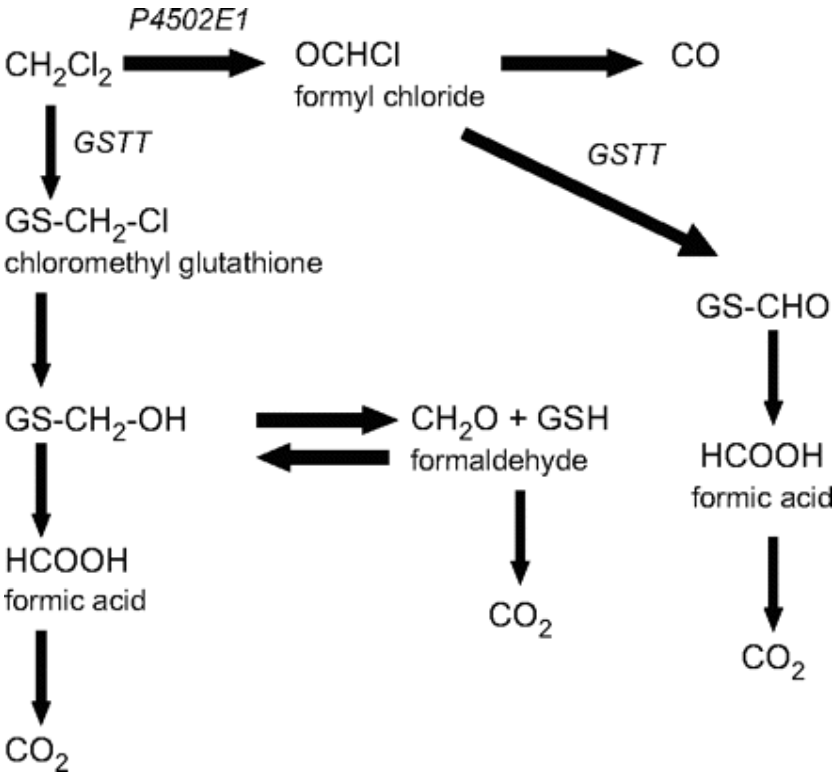

FIG. 1. Biotransformation scheme of methylene chloride (modified after Gargas *et al.*, 1986).

Risk assessment for DCM has mainly been focused on its carcinogenic properties. Carcinogenicity falls within the definition of endpoints for AEGL-2. However, due to a number of uncertainties, the NAC/AEGL committee does not believe that the use of short-term cancer risks is justifiable at present to derive AEGL-2 values (Krewski *et al.*, 2004). It is noted that, where considered appropriate, estimates of the carcinogenic risk are presented in an appendix to the technical support

document (TSD) (as has been done for DCM), but this issue is beyond the scope of the present paper.

Two toxicity endpoints are relevant for AEGL setting for DCM. Human case studies indicate that the main cause of death following a single DCM exposure is related to central nervous system (CNS) depression (Manno *et al.*, 1989, 1992). These effects include loss of consciousness and respiratory depression, resulting in irreversible coma, hypoxia, and death. DCM biotransformation can also give rise to the formation of COHb. COHb levels of up to 30–40% have been reported after incidental exposure to DCM (Langehennig *et al.*, 1976; Manno *et al.*, 1992). This endpoint is especially relevant for patients with severe coronary artery disease; this has been acknowledged for CO for which the AEGL-2 and AEGL-3 values are based on COHb levels in order to protect patients with coronary artery disease (EPA, 2001). Indeed, cardiotoxicity has been reported in a few cases exposed to DCM (Leikin *et al.*, 1990), incidentally leading to death due to cardiac arrest (Stewart and Hake, 1976).

Because of the two different endpoints, specific problems were expected for the derivation of AEGLs. After absorption into the systemic circulation, DCM concentrations in the brain will rise rather quickly. In contrast, the formation of COHb starts slowly but continues as long as the DCM concentrations in blood (liver) are above a specific threshold (Andersen *et al.*, 1991). Maximum COHb levels can sometimes be reached hours after exposure depending both on the concentration and the duration of the exposure (Andersen *et al.*, 1991; Fodor and Roscovanu, 1976; Stewart *et al.*, 1972). It was therefore expected beforehand that the toxicity endpoint of interest might change from CNS depression to COHb formation during an 8-h exposure.

A further issue that had to be addressed is related to the interindividual variation in both pathways. Considerable interindividual differences in activity of P450 2E1 may be present (Snawder and Lipscomb, 2000). Although *CYP2E1* polymorphism has been described, its functional significance appears to be unclear, and it has been suggested that variability in P450 2E1 activity may rather be dominated by environmental and other factors (Haber *et al.*, 2002). The interindividual variation in COHb formation can be attributed to polymorphism of GST, which is a θ-class GST (GSTT1-1) (Bogaards *et al.*, 1993; Mainwaring *et al.*, 1996a,b; Sherratt *et al.*, 1997). This has been well characterized in the human population for which nonconjugators (lacking the GSTT1 enzyme), low conjugators, and high conjugators could be distinguished (Bogaards *et al.*, 1993; Hallier *et al.*, 1994; Thier *et al.*, 1998). The incidence of nonconjugators has been estimated to be between 20 and 60% (Haber *et al.*, 2002; Pemble *et al.*, 1994). The relevance of the absence or presence of the GST pathway was illustrated by Gargas *et al.* (1986), who showed that the COHb level was increased by about 50% in DCM-exposed rats after pretreatment with a GSH depletor.

MATERIALS AND METHODS

Modeling Approach

As described in the introduction, the following issues had to be dealt with in setting AEGL values for DCM.

- Two different relevant toxicity endpoints:

  CNS depression, related to the DCM concentration in brain and COHb formation, via biotransformation to CO.

- A possible change in relevant toxicity endpoint between the 10-min and 8-h exposure:

  CNS effects occur soon after the onset of exposure and peak levels of COHb may be reached hours after cessation of exposure.

- Saturation of the metabolic pathway for CO at a DCM concentration of approximately 500 ppm.
- The presence of GSTT1 polymorphism in humans.

PBPK modeling was considered to be the most appropriate if not the only way to adequately tackle these issues. Several publications deal with the development of PBPK models for DCM. Agency for Toxic Substances and Disease Registry (ATSDR) has summarized and evaluated most of these models including, among others, a model developed by Casanova *et al.* (1996) for DNA-protein cross-link formation in mouse liver (ATSDR, 2000). More recently, the impact of the GSTT1 polymorphism on the carcinogenic risk of DCM has been studied using PBPK modeling (El-Masri *et al.*, 1999; Jonsson and Johanson, 2001; Jonsson *et al.*, 2001). However, these models mainly focus on the carcinogenic potency of DCM and therefore are of little importance within the present context.

In concordance with earlier models for volatile organic compounds in mammals, Andersen *et al.* (1991) described DCM kinetics in terms of inhalatory uptake in the lungs, blood flow–limited distribution of DCM among a "Richly Perfused Organ" compartment, a "Slowly Perfused Organ" compartment, a liver and an adipose tissue compartment. Both metabolic routes, the saturable P450 pathway and the (first order) GST pathway, were thought to occur predominantly in the liver. The P450 pathway finally yields CO, and Andersen *et al.* extended the model with a COHb submodel. This submodel was based on the Coburn-Forster-Kane description of the physiological factors which influence COHb levels in humans (Coburn *et al.*, 1965), with an additional element to account for CO arising from the oxidative metabolism of DCM. This extended model, however, does not contain a separate brain compartment. Reitz *et al.* (1997) extended the earlier PBPK model as developed by Andersen *et al.* with a brain compartment, but did not consider formation of COHb.

For the purpose of AEGL setting, both models were combined in order to calculate in an unambiguous way both DCM concentration in brain and the additional level of COHb resulting from exposure to DCM within one model. Moreover, an implied quadratic equation for the COHb concentration was explicitly solved, and an implied background total amount of CO in blood was explicitly calculated (Supplemental Appendix A1 and Appendix A2, Supplemental Data). The combined PBPK model was implemented into the advanced continuous simulation language computer language (version 11.5.2). Because the AEGL-3 criterion had to be based on rat data, both a human and a rat model were thus developed from original human and rat models, respectively, of Andersen *et al.* (1991) and Reitz *et al.* (1997).

The existence of polymorphism in the GST pathway (conjugators vs. nonconjugators) will mainly affect the formation of CO and not the DCM concentration in blood or brain (see "Sensitivity Analysis") and was accounted for as follows. The stoichiometric yield of CO via formyl chloride was set at 70% in conjugators, whereas the GST route was switched off for nonconjugators, resulting in a 100% stoichiometric yield of CO, resembling nonconjugators. This

was based on Gargas *et al.* (1986) who showed that the COHb level was increased by about 50% in DCM-exposed rats after pretreatment with a GSH depletor, suggesting that in rats about 30% of the formyl chloride is conjugated through GST.

Model Reproducibility Data

The validity of model combination should be checked by reproducing the same model calculations as the original models. Therefore, it was decided to check reproducibility of the model calibration presented in Andersen *et al.* (1991) on data of COHb levels and of DCM concentration in venous blood of six human volunteers during and after exposure to 100 or 350 ppm DCM during 6 h in the same experiment. Andersen *et al.* (1991) also developed a similar model for rats. The rat combined model was reproduced by simulation of the formation of COHb with exposure scenarios of 5159 ppm DCM for 0.5 h and of 1014 ppm DCM for 4 h, as presented by Andersen *et al.* From Reitz *et al.* (1997), a model simulation of DCM concentration in human brain after exposure to 300 ppm DCM during 4 h was chosen to serve the same purpose.

Model Verification Data

The combined model was applied to data obtained from literature for verification and in order to study its general applicability in human and rat studies. It is noted that due to the natural variation in physiological and kinetic parameters within populations, it cannot be expected that a model that is validated for a specific population precisely predicts the results for another group. Indeed, experimental studies were found with a similar experimental setup, but with a different outcome.

Human data obtained by DiVincenzo and Kaplan (1981) were the best described and most suitable. However, it was noted beforehand that at similar exposure levels, DiVincenzo and Kaplan reported lower blood DCM concentrations and COHb levels than Andersen *et al.* (1991). In their study, 11 male and three female nonsmoking volunteers were exposed to 50, 100, 150, or 200 ppm DCM for 7.5 h. Exposures were interrupted after 4.5 h for a half-hour break. All subjects remained sedentary during and after exposure. Data on DCM levels in blood and on COHb levels were reported. DiVincenzo and Kaplan did not provide any physiological details about their volunteers. (It is noted that additional data on these volunteers came to our attention after preparation of the initial draft of this article; Sweeney *et al.*, 2004.) Further interesting data were reported by Åstrand *et al.* (1975). Groups of four or five human subjects were exposed in three series of experiments to either low (250 ppm) or high (500 ppm) DCM concentrations for a total of 140 min (including a 20-min pause without exposure). Subjects were first exposed for 30 or 60 min under resting conditions followed by an exposure at a steady or increasing work load, with the 20-min break of nonexposure between rest and physical exercise. Detailed data on DCM concentrations in arterial and venous blood and of COHb levels in venous blood were presented for only one subject per exposure regimen.

The best-described rat data were obtained from Green *et al.* (1986). Groups of three male F344 rats were exposed to 0, 500, 1000, 2000, or 4000 ppm DCM for up to 6 h. The groups were sacrificed at regular intervals during and after exposure, and levels of COHb and DCM in blood were reported.

No adequate data on DCM levels in human or rat brain were available. Therefore, data on blood DCM concentrations were chosen to be representative of the concentration in brain. The corresponding brain:blood partition coefficient for rats was obtained from Reitz *et al.* (1997).

AEGL Setting

The appropriate dose metrics for AEGL setting are the DCM concentration in human brain (indicative of CNS depression) and the additional COHb formation resulting from DCM exposure. For the present purpose, it suffices to briefly describe the points of departure for the derivation of the respective AEGL values. A detailed substantiation of the appropriate points of departure for CNS depression can be found in the TSD on methylene chloride NAC/AEGL 2005; Technical Support Document on Acute Exposure Guideline Levels (AEGLs) for methylene chloride [Proposed]. A copy can be obtained upon request from Paul S. Tobin, US EPA at Tobin.Paul@epa.gov). This TSD has been discussed, and the AEGL values are adopted as proposed by the NAC/AEGL committee in December 2004. No adequate human or animal data were available that addressed adverse effects associated with the formation of COHb as a result of DCM exposure. However, AEGL-2 and -3 values derived for CO were based on maximum COHb levels; no values were recommended for AEGL-1. Therefore, for reasons of consistency, the criteria that previously had been laid down for the derivation of AEGLs for CO (EPA, 2001) were also applied to DCM.

AEGL-1.

Light-headedness and difficulties with enunciation were reported after a 1-h exposure to 986 ppm or within 15 min of exposure to 868 ppm directly following a 1-h exposure to 514 ppm. No effects that addressed the level of toxicity associated with AEGL-1 were observed in human volunteers exposed to 514 or 515 ppm DCM for 60 min (Stewart *et al.*, 1972). Gamberale *et al.* (1975) exposed subjects for four subsequent 30-min exposure periods to 250, 500, 750, and 1000 ppm. Subjects' assessment of their own well being was slightly better under DCM exposure than under control conditions of nonexposure. The report by Stewart *et al.* (1972) was chosen as the point of departure for the derivation of AEGL-1 levels for DCM. Since no values for AEGL-1 were recommended by the NAC/AEGL committee for CO, only AEGL-1 values for CNS effects were assessed for DCM.

AEGL-2.

Several experimental studies with volunteers have addressed neurobehavioral endpoints that are sensitive subtle effects that may be indicative of more severe effects at higher exposure concentrations but are actually no AEGL-2 effects in themselves. For instance, Gamberale *et al.* (1975) observed no effects on reaction time, short-term memory, or numerical ability in subjects exposed for four subsequent 30-min periods to 250, 500, 750, and 1000 ppm DCM. Putz *et al.* (1979) observed that a 4-h exposure to 195 ppm DCM causes some decreased performance on auditory vigilance task (AVT) and an increase in reaction time to a peripheral light stimulus. Winneke (1974) reported decreased performances in AVT and critical flicker frequency in subjects exposed to 317, 470, or 751 ppm DCM for up to 230 min. However, the results were not always consistent, and no clear concentration-response relation was present. Exposure to 751 ppm for 230 min also induced a diminished performance in some additional tests, but the deviations from control values were small, ranging from approximately 3 to 9%. The effects observed are not considered to be severe enough to cause a serious impairment of escape and, therefore, are regarded as sub-AEGL-2 effects. As to the endpoint of COHb, the AEGL-2 for CO was set at a maximum COHb level of 4%. The NAC/AEGL committee concluded that at this level, patients with coronary artery disease may experience a reduced time until onset of angina (chest pain) during physical exertion. It was further stated that an exposure level of 4% COHb is unlikely to cause a significant increase in the frequency of exercise-induced arrhythmias. An exposure level of 4% COHb was also considered protective of acute neurotoxic effects in children. The maximum level of 4% COHb is therefore also used as a predetermined point of departure for setting AEGL-2 values for DCM, in addition to the background COHb level.

AEGL-3.
No adequate human data addressing the level of toxicity associated with AEGL-3 were available. A number of acute toxicity studies with DCM in different animal species were available, showing a steep dose-response curve for mortality (see NAC/AEGL, 2005. for details). A 4-h exposure to 11,000 ppm was chosen as a no-observed-adverse-effect level for mortality in rats (Haskell Laboratory, 1982). For CO, the AEGL-3 values were based on observations in humans (EPA, 2001). The NAC/AEGL committee concluded that no severe or life-threatening symptoms were observed in healthy subjects showing a COHb level of 40–56%. AEGL-3 values (expressed as air CO concentrations) were calculated starting from a maximum of 40% COHb at the end of exposure and accounting for intraspecies variation. The air concentrations thus derived corresponded to a COHb level of approximately 15%. This level is considered to be additional to the background COHb value. Therefore, DCM exposure should not lead to an increase in the COHb level of more than 15%.

Calculation of AEGL Values
With respect to CNS depression as dose metric, the maximum human brain concentrations corresponding with the respective experimental exposure concentrations that were chosen as points of departure were calculated with the human PBPK model. This maximum brain concentration was then divided by the corresponding uncertainty factor (UF), giving the human norm brain concentration (C_{norm}). For AEGL-3, the corresponding maximum rat brain concentration was calculated with the rat PBPK model. Application of the overall UF gave the human norm brain concentration (C_{norm}). For each AEGL tier, the exposure time T_{exp} is varied, and the corresponding DCM exposure $C_{exp}(T_{exp})$ is calculated with the human PBPK model such that the resulting maximum DCM brain concentration, $C_{brain}(T_{exp}) = C_{norm}$. Thus, when calculating the AEGL-2 value for an exposure duration of 230 min, one should find C_{exp} = 751 ppm. We used an iterative method (chord Newton-Raphson iterations) to automatically find the required relation for a wide range of exposure durations (Supplemental Appendix B, Supplemental Data).

Likewise, for the COHb level as dose metric, given an exposure duration T_{exp} of DCM, the corresponding exposure $C_{exp}(T_{exp})$ is calculated such that the maximum additional COHb ratio in blood is $R_{COHb}(T_{exp}) = R_{criterium}$ (4% for AEGL-2 and 15% for AEGL-3). Care has to be taken in these calculations because, unlike for DCM itself, the maximum COHb level can be reached well after the time of cessation of exposure and the more so the higher the ambient DCM air levels are. The same iterative method as described above is used for these calculations. To account for polymorphism, these calculations were performed both for "conjugators" and for "nonconjugators". During the iterations, PBPK model calculations were performed for determining the DCM brain concentration and the blood COHb level.

Sensitivity Analyses
Sensitivity analyses were performed in order to study the dependency of the estimated AEGL values on the accuracy of the individual model parameters. The dependence of DCM and COHb in blood during and after exposure to DCM on body weight, alveolar ventilation, V_{max} for the P450 pathway, yield factor (fraction of formyl chloride converted into CO), and rate of GSH conjugation (k_f) was assessed. The analysis concerns the time course of the fractional variation in concentration ($\Delta C/C$) due to a fractional variation in the value of one of these parameters ($\Delta V_{max}/V_{max}$, $\Delta k_f/k_f$, etc.). Positive values indicate fractional increase, and negative values indicate fractional decrease of the concentration due to an increase of the parameter value. Analysis was carried out for two exposure scenarios, i.e., exposure to 50 and 200 ppm DCM during 8 h.

RESULTS

Model Reproducibility

The combination of the models of Andersen *et al.* (1991) and Reitz *et al.* (1997) has been implemented in two ACSL codes, one human model and one rat model. These as well as the list of the model parameters used can be found in Supplemental Appendix C (Supplemental Data). The richly perfused tissue group and brain compartment are as in Reitz *et al.* (1997), while the COHb submodel has been modified according to Supplemental Appendix A (Supplemental Data).

Running this PBPK model with the parameterization used by Andersen *et al.* (1991) for COHb and DCM concentration in venous blood, similar results were obtained (data not shown, see NAC/AEGL, 2005). With respect to the COHb model, this indicates that the explicit calculation of the COHb level and the background CO total amount in blood did not introduce new errors. The original parameterization (e.g., setting parameters for the brain compartment to zero) as in Andersen *et al.* (1991) was used for these calculations. Likewise, running our PBPK model using the original parameterization of Reitz *et al.* (1997) for DCM in rat brain, similar results were obtained (data not shown, see NAC/AEGL, 2005).

Model Verification

Figure 2 shows the measured values for COHb and DCM in human blood of DiVincenzo and Kaplan (1981), together with the model simulations.

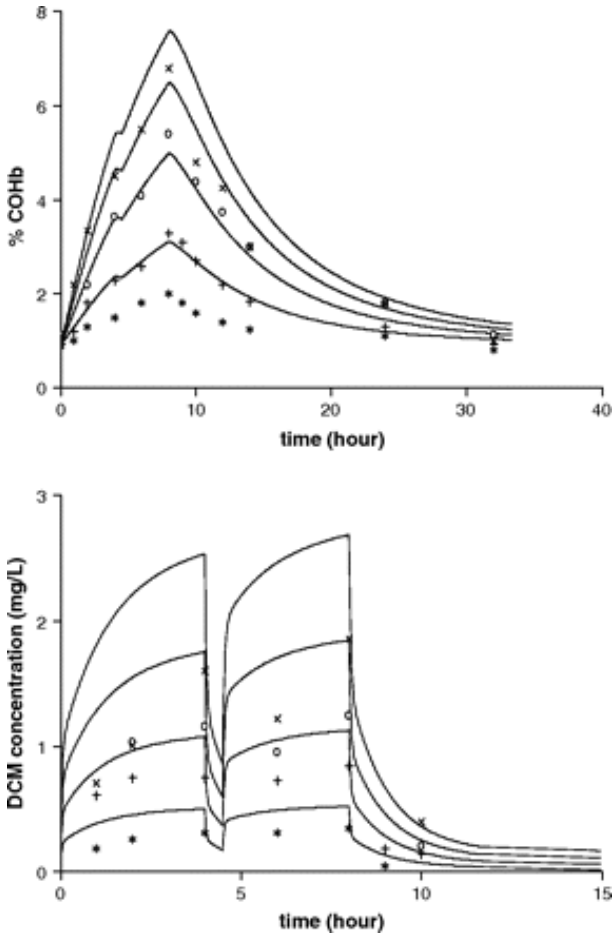

FIG. 2. Upper panel: PBPK simulation of COHb in blood in humans exposed for 7.5 h, with a half-hour break after 4.5 h, to 50 (*), 100 (+), 150 (○), or 200 ppm (x). Data represent averages of four to six individuals. Experimental values appear to be overestimated by a factor of 1.1 (highest exposure concentration) to 1.5 (lowest exposure concentration). Lower panel: PBPK simulation of DCM in blood in the same subjects. Experimental values appear to be overestimated by about 50% at most.

Figure 3 shows the modeling simulations based on one of the experiments by Åstrand *et al.* (1975). Data are shown for one subject exposed to 500 ppm for 30 min at rest, followed by a 20-min break of nonexposure, and subsequently exposed during three 30-min periods with increasing exercise on a bicycle ergometer (50, 100, and 150 W, respectively).

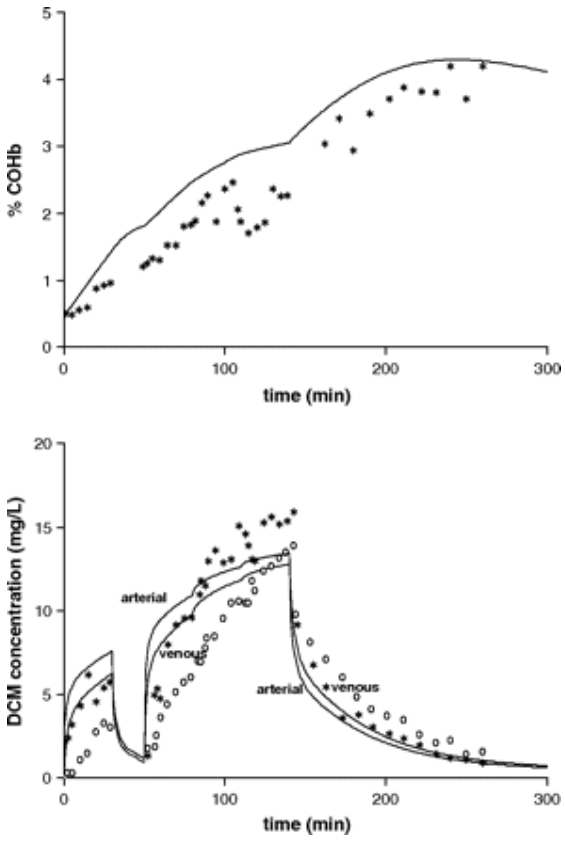

FIG. 3. Upper panel: PBPK simulation of COHb in blood in a single subject exposed to 500 ppm for 30 min at rest, followed by a 20-min break of nonexposure, and subsequently exposed during three 30-min periods with increasing exercise on a bicycle ergometer (50, 100, and 150 W). Lower panel: PBPK simulation of DCM in arterial and venous blood in the same subject.

Figure 4 shows the measured values for COHb and DCM in rat blood of Green *et al.* (1986), together with the model simulations.

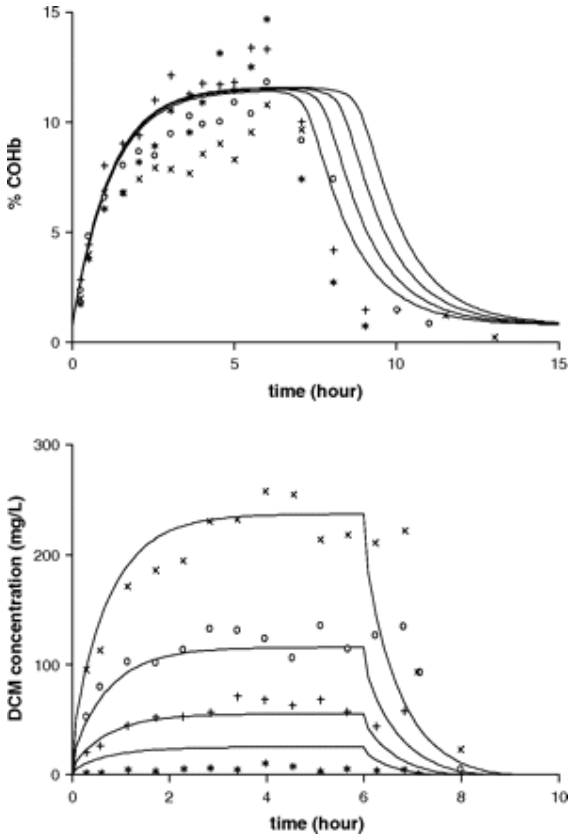

FIG. 4. Upper panel: PBPK model simulation of COHb in blood in rats exposed for 6 h to 500 (*), 1000 (+), 2000 (○) and 4000 (x) ppm DCM. Data (*, +, ○, x) represent average values of three rats. Note that the maximum observed COHb level in rats is about 13–14% due to saturation of the biotransformation pathway. Lower panel: PBPK model simulation for corresponding DCM concentrations in blood.

Calculation of AEGL Values

Krewski *et al.* (2004) describe the general procedure and methodology of deriving AEGLs, including the selection of appropriate UFs. Table 1 summarizes the AEGL values as calculated with the present PBPK model.

TABLE 1 Proposed AEGL-1, -2, and -3 values (ppm) as calculated with the present PBPK model

	Exposure duration				
Classification	10 min	30 min	1 h	4 h	8 h
AEGL-1 (Nondisabling)					
CNS effects	**290**	**230**	**200**	**NA**[a]	**NA**[a]
AEGL-2 (Disabling)					
CNS effects	**1700**	**1200**	1000	740	650
COHb (nonconjugators)	4600	1400	**560**	**100**	**60**
AEGL-3 (Lethal)					
CNS effects	**12,000**	**8500**	**6900**	**4900**	4200
COHb (nonconjugators)	160,000	52,000	25,000	5300	**2100**

Note. The AEGL values are given for individual endpoints; the final values adopted by the NAC/AEGL committee as proposed values are presented in bold. [a] NA: Not applicable since these values would be above the corresponding AEGL-2 values.

AEGL-1.
Using the human PBPK model, the maximal DCM concentration in human brain following a 1-h exposure to 514 ppm (Stewart *et al.*, 1972) was calculated to be 0.063mM. Since susceptibilities for gross CNS-depressing effects are considered not to vary by more than a factor 2–3, an intraspecies UF of 3 was considered sufficient, resulting in a maximum target concentration of DCM in the human brain of 0.021mM. The human PBPK model was subsequently used to calculate the DCM concentrations in environmental air for exposures of up to 8 h that will result in a maximum brain concentration of 0.021mM. Figure 5 shows the calculation result for AEGL-1 values.

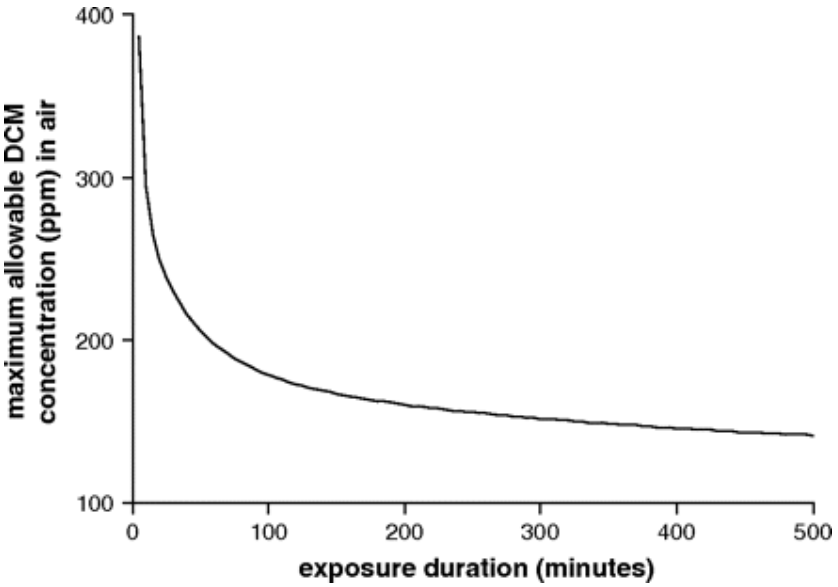

FIG. 5. Maximum allowable DCM concentration in ambient air equivalent to a maximum target DCM concentration of 0.021mM in human brain.

AEGL-2.
Starting from the 230-min exposure to 751 ppm (Winneke, 1974), the equivalent maximum DCM concentration in brain was estimated to be 0.137mM using the human PBPK model. The toxic effects of DCM studied in the relevant experiments are less severe than those defined for AEGL-2. The CNS effects observed at 751 ppm are very mild, and this exposure concentration is far below concentrations that would impair the ability to escape. Taking all the relevant data into consideration, application of an intraspecies factor of > 1 would lead to CNS-based AEGL-2 values that would conflict with these data. For instance, the physical performance of volunteers exposed under physical exertion, appeared not to be seriously impaired when exposed to 500 ppm for 2 h (work load up to 150 W) or to 750 ppm for 1 h (work load of 50 W) (Åstrand *et al.*, 1975; Engström and Bjurström, 1977). Therefore, application of an intraspecies UF of 1 is considered sufficient, resulting in a maximum target concentration of DCM in human brain of 0.137mM. The human PBPK model was subsequently used to calculate the DCM concentrations in environmental air for exposures of up to 8 h that will result in a maximum brain concentration of 0.137mM. In addition, the human PBPK model was used to calculate the DCM concentrations corresponding to a maximum additional COHb level of 4%, both for conjugators and nonconjugators (Fig. 6). Nonconjugators (subjects deficient in GSTT1) reach the maximum additional COHb level of 4% at lower DCM concentrations than conjugators. For nonconjugators, the relevant toxic endpoint changes from CNS depression to COHb formation between 30 and 60 min of exposure. The difference

in DCM brain concentration between conjugators and nonconjugators appeared to be negligible (data not shown).

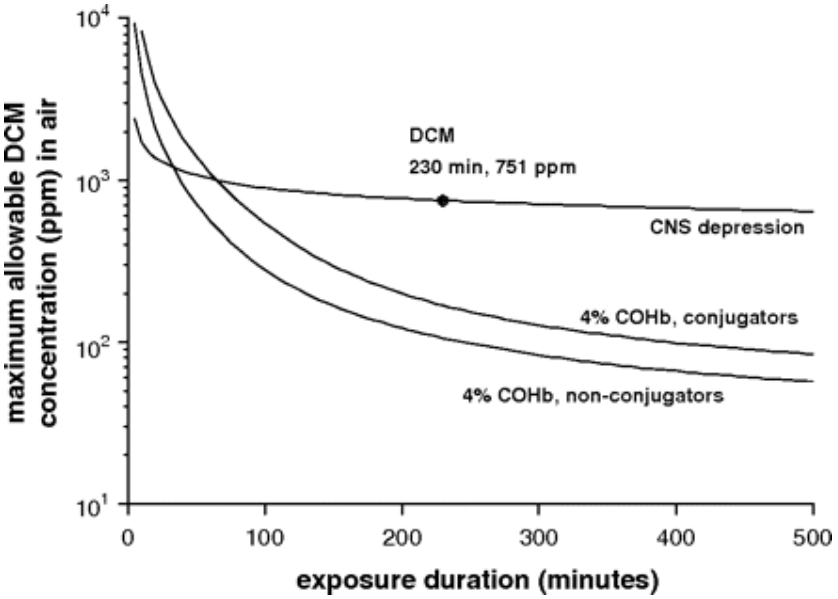

FIG. 6. Maximum allowable DCM concentrations in ambient air equivalent to a maximum target DCM concentration of 0.137mM in human brain based on an exposure regimen of 230 min to 751 ppm DCM (symbol: reference exposure regimen) or to a maximum additional COHb level of 4%.

AEGL-3.

The point of departure for the estimation of the maximum DCM concentration in brain was a 4-h exposure to 11,000 ppm at which no mortality was observed in rats. Using a rat PBPK model, it was estimated that this exposure scenario would correspond to a maximum DCM concentration in rat brain of 3.01mM. Since the interspecies extrapolation was performed with PBPK modeling and the differences in susceptibility between animal species appeared to be very small, an interspecies UF of 1 was considered adequate. An UF of 3 was applied to account for the interindividual variation regarding mortality caused by CNS depression, resulting in a maximum DCM concentration in human brain of 1.0mM. The human PBPK model was subsequently used to calculate the DCM concentrations in environmental air for exposures of up to 8 h that will result in a maximum brain concentration of 1.0mM. In addition, the human PBPK model was used to calculate the DCM concentrations corresponding to a maximum additional COHb level of 15%, both for conjugators and nonconjugators. However, due to the saturation of the P450 pathway, the formation of COHb did not reach the level of 15% in conjugators. For nonconjugators, the relevant toxic endpoint changes between 4 and 5 h of exposure from CNS depression to COHb formation (Fig. 7).

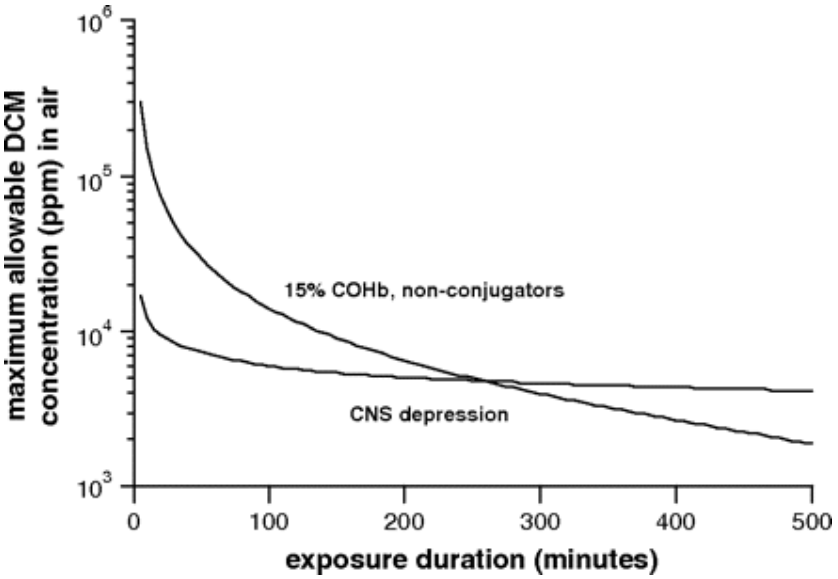

FIG. 7. Maximum allowable DCM concentrations in ambient air equivalent to a maximum target DCM concentration of 1.0mM in human brain (based on an exposure regimen of 4 h to 11,000 ppm DCM in rats, total UF = 3) or to a maximum additional COHb level of 15%.

Sensitivity Analyses

The sensitivity of DCM and COHb level appeared to be quite different. Furthermore, the sensitivity depends on exposure level, be it not strongly. The results of the analysis should be interpreted with care, as sensitivity generally also depends on the nominal values of the parameters that are varied.

For DCM in blood, not only the sensitivity during exposure to variation in yield is *nihil*, which is obvious, but also the sensitivity to variation in rate of GSH conjugation, indicating that this pathway is marginal with respect to the P450 pathway. Sensitivity to variation in body weight is nil to marginal during exposure and increases thereafter, when DCM concentration in blood decreases rapidly to zero. Blood levels of DCM appear to be sensitive to variation in the maximum P450 metabolism rate and the alveolar ventilation rate. Increasing the rate of metabolism decreases the DCM levels with a relative sensitivity of 0.3 (50 ppm DCM) to 0.5 (200 ppm DCM) at the end of the exposure period, while it increases DCM levels due to increasing alveolar ventilation with a maximum relative sensitivity of about 0.8, independent of the exposure level, at the start of exposure.

For COHb, as for DCM, the sensitivity to variation in rate of GSH conjugation is almost nil. The sensitivity to variation in the yield factor is positive with a maximum of 0.7 (50 ppm DCM) to 0.85 (200 ppm DCM). Also, increasing the maximum rate of P450 metabolism will increase COHb levels but less than when increasing the yield factor: maximum relative sensitivity ranges from 0.2 (50 ppm DCM) to 0.6 (200 ppm DCM). Increasing body weight will reduce COHb level during exposure with a relative sensitivity of about 0.2, independent of exposure level. The sensitivity of COHb level to alveolar ventilation is negative, due to the increasing sink caused by ventilation of CO out of the body. Nevertheless, the increasing uptake rate of DCM with increasing ventilation rate combined with saturation of the P450 pathway will decrease the relative sensitivity during exposure.

DISCUSSION

Since AEGLs are to be set for single exposures, over 10–480 min of exposure, and for three different levels of toxicity, PBPK models can be of great value (Bruckner *et al.*, 2004). PBPK modeling provides the opportunity to make adequate use of all information available and to found the assessment on the appropriate tissue dose metrics. In the case of DCM, two different toxicity endpoints were identified with a change in relevant endpoint within the relevant exposure time of 10 min to 8 h. Additional topics that had to be encountered included saturation of the P450 pathway, the variability in P450 2E1 activity (Haber *et al.*, 2002), and polymorphism for GSTT1 (Bogaards *et al.*, 1993; Hallier *et al.*, 1994; Thier *et al.*, 1998). A relatively high P450 2E1 activity would lead to an increased formation of CO and hence of COHb but simultaneously to lower tissue concentrations of DCM itself. As to the polymorphism of GSTT1-1, nonconjugators (lacking the GSTT1-1 enzyme), low conjugators, and high conjugators have been identified in the human population (Bogaards *et al.*, 1993; Hallier *et al.*, 1994; Thier *et al.*, 1998). The incidence of nonconjugators has been estimated to be between 20 and 60% (Haber *et al.*, 2002; Pemble *et al.*, 1994). The fact that the COHb level was increased by about 50% in DCM-exposed rats after pretreatment with a GSH depletor (Gargas *et al.*, 1986) might point to 50% higher COHb levels in human nonconjugators (lacking GSTT1) as compared with conjugators. The presence of GST polymorphism in humans is therefore of great relevance for the estimation of the COHb formation due to DCM exposure.

The stoichiometric CO yield was set in accordance with the value of 0.71 given by Andersen *et al.* (1991) that was probably based on the findings by Gargas *et al.* (1986). Assuming that in GSH-depleted rats 100% of the formyl chloride is converted to CO, it was estimated that in "normal" rats 70% of the formyl chloride is converted to CO and subsequently to COHb. To account for the GSTT1 polymorphism in humans, it was reasoned that homozygotous conjugators could be represented by a CO yield of 70%, while nonconjugators were represented by a yield of 100%.

The final PBPK model, including the general parameter setting, was a combination of models that had been peer reviewed and used for specific risk assessments by U.S. EPA (2002) and ATSDR (2000). Both the COHb formation as well as the DCM concentration in brain could be simulated within one model. The results published by Andersen *et al.* (1991) and Reitz *et al.* (1997) could be reproduced, indicating that combining the two models did not affect the outcome.

The combined model was verified with the data obtained with volunteers by Åstrand *et al.* (1975) and by DiVincenzo and Kaplan (1981). One should keep in mind that due to the natural variation in physiological and kinetic parameters within populations, it would be improper to expect that a model that is validated for a specific population precisely predicts the results for another group. Unfortunately, Åstrand *et al.* (1975) reported detailed data on only a single subject per series of experiments; data on group averages were limitedly reported. Therefore, the model predictions of the COHb levels, although somewhat higher, were found to be within reasonable limits considering the human interindividual variation. As to the DCM concentrations in arterial and venous blood, the initial increase was somewhat overestimated by the model, but the pattern and the maximal concentrations were predicted reasonably well. Simulations of the other exposure regimens by Åstrand *et al.* (1975) showed comparable results (data not shown). The predicted patterns of the DCM concentration in blood and the COHb level over time as reported by DiVincenzo and Kaplan (1981) appeared to be accurate. Our model overestimated the DCM

blood concentration and the COHb level by 50% at the most. These results are satisfactory considering that the human interindividual variation generally is much larger. Recently, Sweeney *et al.* (2004) estimated individual values for V_{max} for the P450 pathway and yield factor for CO based on the individual data for age, height, and body weight for the 13 volunteers from the DiVincenzo and Kaplan study. They found CO yields that are considerably lower than the 0.71 used by Andersen *et al.* (1991). However, instead of optimizing the V_{max} and the yield factor in combination, Sweeney *et al.* first fitted the V_{max} and subsequently the CO yield for each individual. Since the parent compound concentrations and the COHb levels are related within an individual, this will have influenced their results. This is confirmed by our sensitivity analyses; a threefold higher V_{max} (i.e., the range in V_{max} as reported by Sweeney *et al.*, 2004) would have not only resulted in approximately 1.5 times lower DCM concentrations in blood but also in an approximately 50% higher COHb level at an exposure concentration of 200 ppm. It is therefore more appropriate to consider combinations of parameter settings rather than settings for single parameters. This is further illustrated by the study by Stewart *et al.* (1972) who found maximum COHb levels of about 7–9% in two volunteers but 15% in one volunteer exposed to 986 ppm DCM for 2 h. Andersen *et al.* (1991), when trying to fit his PBPK model to these data, assumed a fivefold difference in V_{max} between the individuals in order to obtain proper individual fits. However, the high level of COHb could also be the result of a combination of a slightly higher V_{max} in combination with a 100% yield, assuming the subject lacked the GSTT1 enzyme (data not shown). The validation of our model against the data from Åstrand *et al.* (1975) and from DiVincenzo and Kaplan (1981) shows that the model parameters used are adequate for the present purpose.

It was shown that the present PBPK model also adequately predicted COHb levels and blood concentrations of DCM in rats exposed to DCM concentrations of up to 4000 ppm for 6 h or to 5159 ppm for 30 min. Since the metabolism in rats and humans appears to be very similar (ATSDR, 2000; "NAC/AEGL, 2005), the human PBPK model is considered adequate for high exposure concentrations as well. By using both a rat and a human PBPK model, the uncertainties involved in the extrapolation of toxicity data across species can be significantly reduced.

The adequacy of the AEGL values (Table 1) derived with the PBPK model is further substantiated by additional human data that support the present values. For instance, no neurobehavioral effects as defined by the AEGL-2 were observed in volunteers exposed to concentrations of up to a 1000 ppm with or without physical exertion (Åstrand *et al.*, 1975; Engström and Bjurström, 1977; Gamberale *et al.*, 1975) or in workers exposed to a 15-min time-weighted average concentration of up to 1700 ppm or to an 8-h TWA exposure of up to 969 ppm (Moynihan-Fradkin, 2001).

AEGLs serve a similar purpose as Emergency Response Planning Guidelines (ERPGs) derived by the American Industrial Hygiene Association. ERPGs are also set at three different levels comparable to the AEGL-levels, but are set for only 1 h of exposure. The calculated 1-h AEGL-1, -2, and -3 values of 200 ppm, 560 ppm, and 6900 ppm, respectively (Table 1), are comparable with the corresponding ERPG-1, -2, and -3 values for DCM of 200 ppm, 750 ppm, and 4000 ppm, respectively (AIHA, 2005).

Although relevant for AEGL setting, data expressing the quantitative relationship between concentration, time, and specific toxicity endpoints are seldom available. In the absence of such data, extrapolation over time is performed using Haber's rule $C^n \times t = k$ with conservative default values for n. This approach is associated with often unknown uncertainties. It is shown that PBPK modeling is of great use

to properly perform time extrapolations from 10 min to 8 h based on the appropriate dose metrics. In addition, the change in toxic endpoint of interest in the case of DCM hereby could be sufficiently accounted for. Further, by using both a rat and a human model, several uncertainties associated with interspecies extrapolation for AEGL-3 are adequately taken into account. Specific aspects determining the interindividual variation (e.g., GSTT1 polymorphism) could be addressed. The AEGL values as calculated with the present PBPK model are substantiated by human data, showing that these values are effective. It is noted that interesting developments are going on as to the topic of modeling interindividual variation. For instance, the approach taken by Price *et al.* (2003), to make use of records of individual physiological parameters for actual individuals in order to model interindividual variation, is very interesting within the present context.

SUPPLEMENTAL DATA

Supplemental Appendices A, A1, A2, B, and C and supplementary data are available online at www.toxsci.oxfordjournals.org.

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