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Revised assessment of cancer risk to dichloromethane II. Application of probabilistic methods to cancer risk determinations

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Abstract

An updated PBPK model of methylene chloride (DCM, dichloromethane) carcinogenicity in mice was recently published using Bayesian statistical methods (Marino et al., 2006). In this work, this model was applied to humans, as recommended by Sweeney et al. (2004). Physiological parameters for input into the MCMC analysis were selected from multiple sources reflecting, in each case, the source that was considered to represent the most current scientific evidence for each parameter. Metabolic data for individual subjects from five human studies were combined into a single data set and population values derived using MCSim. These population values were used for calibration of the human model. The PBPK model using the calibrated metabolic parameters was used to perform a cancer risk assessment for DCM, using the same tumor incidence and exposure concentration data relied upon in the current EPA (1991) IRIS entry. Unit risks, i.e., the risk of cancer from exposure to 1 μg/m^3 over a lifetime, for DCM were estimated using the calibrated human model. The results indicate skewed distributions for liver and lung tumor risks, alone or in combination, with a mean unit risk (per μg/m^3) of 1.05×10^{-9}, considering both liver and lung tumors. Adding the distribution of genetic polymorphisms for metabolism to the ultimate carcinogen, the unit risks range from 0 (which is expected given that approximately 20% of the US population is estimated to be nonconjugators) up to a unit risk of 2.70×10^{-9} at the 95th percentile. The median, or 50th percentile, is 9.33×10^{-10}, which is approximately a factor of 500 lower than the current EPA unit risk of 4.7×10^{-7} using a previous PBPK model. These values represent the best estimates to date for DCM cancer risk because all available human data sets were used, and a probabilistic methodology was followed.

Keywords: Methylene chloride; Dichloromethane; Risk assessment; PBPK modeling; Bayesian analysis; Monte Carlo analysis; GST polymorphism

1. Introduction

Dichloromethane (DCM, methylene chloride) is the industrial solvent of choice for cellulose acetate production, with uses in consumer products such as paint strippers and in the decaffeinating process of coffee. In response to questions about the long-term effects of exposure to DCM, several chronic toxicity/oncogenicity studies were conducted in the late 1970s. Lung tumors were observed in mice exposed to DCM by inhalation, and liver tumors were observed in mice exposed to DCM by inhalation or per os.

The roles of cytochrome P450 (CYP) and glutathione *S*-transferase (GST) in the metabolism of DCM were recognized as key to the development of tumors in experimental animals (reviewed in Slikker et al., 2004a,b). According to this MOA, carcinogenicity in the liver and lungs of mice is dependent upon a dose-dependent transition in metabolism from a cytochrome P450 enzyme pathway (CYP 2E1) to a glutathione *S*-transferase (GST-T1) pathway. The CYP2E1 oxidation enzymes have a high affinity for DCM; thus, this pathway predominates at low concentrations

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resulting in the formation of carbon monoxide (among other metabolites) that binds to hemoglobin to form carboxyhemoglobin. This pathway is saturable as the exposure or dose is increased, shifting the metabolism of DCM to the GST pathway that has a lower affinity but higher capacity. It is at concentrations above which this shift occurs that an increase in tumors is observed in laboratory animals. Because of the dose-dependent change in metabolism (and number of tumors), the risk of carcinogenesis from exposure to DCM is non-linear (Slikker et al., 2004a).

Using this hypothesis, a PBPK model was developed for DCM by Andersen et al. (1987) to examine the importance of these metabolic pathways for tumor formation in laboratory animals and the potential implication in humans. The US Environmental Protection Agency (EPA) first estimated the risk of cancer from lifetime exposure to an airborne concentration of $1\,\mu g/m^3$ DCM using a linear-multistage approach from the results of the National Toxicology Program (NTP) chronic study. Assuming that no thresholds for carcinogenesis exist, that DCM is not a genotoxic carcinogen, and that humans are more susceptible to cancer than are rodents (EPA, Guidelines for Carcinogen Risk Assessment 1986), the risk of cancer from 70 years of breathing $1\,\mu g/m^3$ DCM (assuming a daily air exchange of $20\,m^3$) was calculated to be 4.1×10^{-4} (EPA, 1985). However, following the formulation of the PBPK model of Andersen et al. (1987), the EPA adopted the use of PBPK modeling for DCM in 1991 as a reasonable means for evaluating risk by predicting target organ doses in the species of interest, and recalculated the unit risk value to be 4.7×10^{-7} (EPA, 1991). Since then, several quantitative assessments of DCM cancer risk, using the basic Andersen et al. model, have incorporated advances in scientific understanding of metabolism and statistical approaches to address variability in humans. For example, information on human GST-T1 polymorphisms was incorporated in PBPK modeling using probabilistic (Bayesian) methodology (El-Masri et al., 1999; Jonsson and Johanson, 2001). These advances have provided a means of incorporating population distributions that better reflect the likely real-world situation (Portier and Kaplan, 1989). This model for animals has recently been updated using new data for CYP-associated metabolism and using Bayesian statistics (Marino et al., 2006).

Recent models (Casanova et al., 1996; El-Masri et al., 1999; Jonsson and Johanson, 2001) have utilized DNA-protein cross-links (DPX) as dosimeters, rather than the more traditional dosimeter of mg DCM metabolized by the GST pathway/L tissue/day, and Bayesian models were calibrated on a single human exposure data set (El-Masri et al., 1999; Jonsson and Johanson, 2001). While both dose metrics are related to GST metabolism of DCM, DPX has only been demonstrated in mouse liver, not in mouse lung (the other target organ) and not in human liver (Casanova et al., 1996, 1997). Thus, extrapolation to the lung and humans has to be performed relative to metabolism by GST pathways in those tissues without the actual confirmation of a measurable result. Of course, the argument that DPX is at least measurable in one tissue, compared with the proposed reactive chloromethylglutathione metabolite proposed in the Andersen et al. (1987) model, is an advantage (Liteplo et al., 1998); on the other hand, DPX assumes that formaldehyde is the reactive metabolite, a hypothesis for which there are fewer supporting data (Wheeler et al., 2001). Furthermore, the previous risk assessments have used limited data sets, and—in only one case—individual data from human subjects (Jonsson and Johanson, 2001). A recent publication by Sweeney et al. (2004) provided individual data for human subjects used by DiVincenzo and Kaplan (1981) to estimate group-mean kinetic parameters. These recent publications have led us to consider revising the cancer risk assessment by incorporating all available human exposure data sets in a Bayesian analysis. The previous paper by Marino et al. (2006) reported an improved mouse PBPK model for the traditional dosimeter for DCM of mg DCM metabolized by the GST pathway/L tissue/day using newer animal data and Bayesian statistics. We report here the results of calibration of that model with several studies of human volunteers (Åstrand et al., 1975; DiVincenzo and Kaplan, 1981; Engström and Bjurström, 1977; Stewart et al., 1972) and the estimation of unit risk factors using MCMC methodology.

2. Methods

2.1. Model structure

The PBPK model structure used for this analysis is the basic structure developed by Andersen et al. (1987, 1991) and refined by Marino et al. (2006) for the mouse, with a revision for humans to include extrahepatic/extrapulmonary metabolism (Fig. 1), as suggested by Sweeney et al. (2004). The model describes metabolism of DCM in both the liver and the lung by two competing pathways, an oxidative pathway and a glutathione conjugation pathway. The P450 (oxidative) pathway is described with saturable

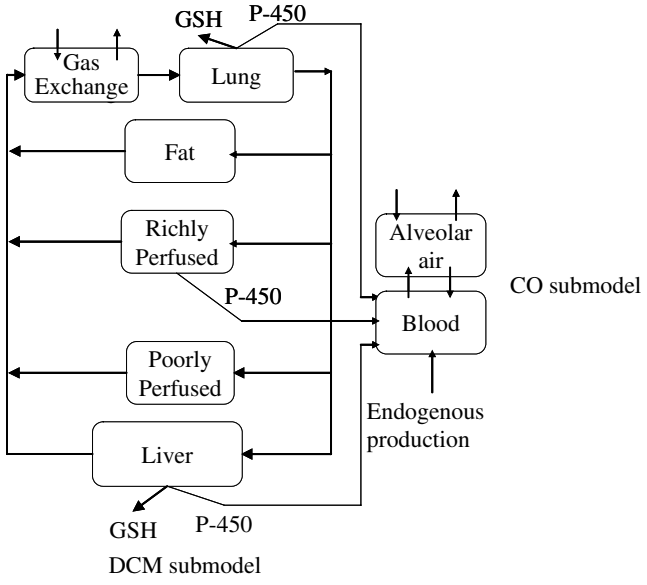

Fig. 1. PBPK model modified from Sweeney et al. (2004).