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FORUM

Workshop Overview: Reassessment of the Cancer Risk of Dichloromethane in Humans

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The U.S. Environmental Protection Agency (U.S. EPA) classifies dichloromethane (DCM) as a "probable human carcinogen," based upon its risk assessment conducted in the late 1980s (http://www.epa.gov/iris/subst/0070.htm). Since that time, cancer risk-assessment practices have evolved, leading to improved scientifically based methods for estimating risk and for illuminating as well as reducing residual uncertainties. A new physiologically based pharmacokinetic (PBPK) model has been developed, using data from human volunteers exposed to low DCM levels, that provides new information on the human to human variability in DCM metabolism and elimination (L. M. Sweeney *et al.*, 2004, *Toxicol. Lett.* 154, 201–216). This information, along with data from other published human studies, has been used to develop a new cancer risk estimation model utilizing probabilistic methodology similar to that employed recently by U.S. EPA for other chemicals (ENVIRON Health Sciences Institute, 2005, Development of population cancer risk estimates for environmental exposure to dichloromethane using a physiologically based pharmacokinetic model. Final Report to Eastman Kodak Company). This article summarizes the deliberations of a scientific peer-review panel convened on 3 and 4 May 2005 at the CIIT Centers for Health Research in Research Triangle Park, North Carolina, to review the "state of the science" for DCM and to critically evaluate the new information for its utility in assessing potential human cancer risks from DCM exposure. The panel (Melvin E Andersen, CIIT Centers for Health Research, Research Triangle Park, NC 27709; A. John Bailer, Miami University, Scripps Gerontology Center, Oxford, OH 45056; Kenneth S. Crump, ENVIRON Health Sciences Institute, Ruston, LA 71270; Clifford R. Elcombe, University of Dundee, Biomedical Research Centre, Dundee DD1 9SY, United Kingdom; Linda S. Erdreich, Exponent, 420 Lexington Avenue, Suite 1740, New York, NY 10170; Jeffery W. Fisher, University of Georgia, Department of Environmental Health Science, Athens, GA 30602; David Gaylor, Gaylor and Associates, LLC, Eureka Springs, AR 72631; F Peter Guengerich, Vanderbilt University, Department of Biochemistry, Nashville, TN 37232; Kenneth Mundt, ENVIRON Health Sciences Institute, Amherst, MA 01004; Lorenz R Rhomberg, Gradient Corporation, Cambridge, MA 021138; Charles Timchalk, Pacific Northwest National Laboratory, Richland, WA 99352), chaired by M.E.A., was composed of experts in xenobiotic metabolism and carcinogenic mechanisms, PBPK modeling, epidemiology, biostatistics, and quantitative risk assessment. Observers included representatives from U.S. EPA, CIIT, and Eastman Kodak Company (Kodak), as well as several consultants to Kodak. The workshop was organized and sponsored by Kodak, which employs DCM as a solvent in the production of imaging materials. Overall, the panel concluded that the new models for DCM risk assessment were scientifically and technically sound and represented an advance over those employed in past assessments.

Key Words: **dichloromethane; cancer; PBPK; epidemiology; risk assessment.**

The workshop was structured around four key topics: (1) the role of dichloromethane (DCM) metabolism in the mode of carcinogenic action, (2) physiologically based pharmacokinetic (PBPK) modeling, (3) use of Bayesian (Markov Chain Monte Carlo [MCMC]) estimation methods in a probabilistic risk assessment for DCM, and (4) some recent findings from occupational cohort mortality studies of DCM-exposed workers and case-control studies of specific cancers. For each of these topics, there was an introductory presentation by an invited speaker (Michael L. Gargas, Sapphire Group, Dayton, OH 45431; Lisa M. Sweeney, Sapphire Group, Dayton, OH 45431; Harvey J. Clewell, CIIT Centers for Health Research, Research Triangle Park, NC 27709; Karl E. Wende, Eastman Kodak Company, Health Safety & Environment, Rochester, NY 14650) and a panel discussion framed around a series of charge questions, which are described below.

Panel members and observers raised a number of questions and comments dealing mainly with (1) the modifications to previous PBPK models for DCM that were incorporated into the present work, (2) the impact of the evolving DCM science

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on quantitative risk-assessment issues, and (3) the completeness of information included in the technical report for the probabilistic risk-assessment project. These questions and comments are summarized below.

Role of DCM Metabolism in Mode of Carcinogenic Action (Michael L. Gargas)

Abundant experimental data have established that DCM is metabolized by cytochrome P450 (CYP) to carbon monoxide (CO) and by glutathione transferases (GSTs) to formaldehyde (Anders *et al.*, 1977; Kubic and Anders, 1975). Additional studies have shown that CYP2E1 catalyzes the biotransformation of DCM to CO (Kim and Kim, 1996) and that GST theta (GSTT1-1) catalyzes the biotransformation of DCM to formaldehyde (Ahmed and Anders, 1978; Anders *et al.*, 1977; Blocki *et al.*, 1994). Moreover, the CYP2E1 pathway is a high-affinity, low-capacity pathway that becomes saturated at relatively low substrate concentrations, whereas the GSTT1-1 pathway is a low-affinity, high-capacity pathway that predominates at high exposure levels (Anders *et al.*, 1977; Gargas *et al.*, 1986). Hence, during chronic exposures to high airborne DCM concentrations, the GSTT1-1 pathway is the primary catalyst of DCM metabolism because the CYP2E1 pathway is saturated.

The flux through both the CYP and GST pathways is substantially greater in the mouse than in humans and rats. A significant carcinogenic response is seen in the liver and lungs of female B6C3F1 mice, but not in F-344 rats, and these tumors are seen only at high exposure concentrations ($\geq$ 2000 ppm) (NTP, 1986), where the CYP pathway is saturated. These observations lead to the inference that DCM is bioactivated to a carcinogenic intermediate by the GSTT1-1 pathway. Moreover, because GSTT1-1 activity is much higher in mouse tissues than in human tissues, it is presumed that far less GST-catalyzed bioactivation of DCM occurs in humans than in mice. There is no experimental evidence indicating that pathways other than the CYP and GST pathways catalyze the biotransformation of DCM to carcinogenic intermediates. Hence, the CYP and GST pathways appear to be the most important pathways for determining the internal dosimetry of target tissues and dose-response relationships (Andersen *et al.*, 1987; Green, 1983).

In vivo studies in mice and *in vitro* studies with both mouse and human tissues provide kinetic data that support the biological plausibility of a dose-dependent transition from CYP- to GST-dominated metabolism with increasing DCM exposure concentrations and the presumption that only the GST-dependent metabolism of DCM plays a mechanistic role in tumor formation in mice. Three alternative dose metrics have been considered previously for assessing the carcinogenicity of DCM: Area Under the tissue concentration Curve (AUC_{tissue}) of DCM itself, time-integrated production of CYP pathway metabolites, and time-integrated production of GST pathway metabolites. The modest chemical reactivity of DCM appears to rule out any significant role for the parent chemical. Moreover, the dose dependency of the mouse tumor response in the face of saturated kinetics of DCM biotransformation via the CYP pathway makes this pathway unlikely. Finally, the GST-dependent pathway is consistent with the mutagenicity of DCM, the high-dose kinetics of DCM metabolism, and the mouse tumor response to DCM exposure (Andersen *et al.*, 1987; Green, 1983).

Charge questions to the panel were:

- Is there agreement that the CYP and GST pathways are the most important for dosimetry and risk assessment?
- There was general agreement by the panel that these two pathways are the most important pathways for dosimetry and risk assessment. The panel acknowledged that more information was needed regarding the potential impacts on risk of GSTT1-1 polymorphisms and the potential variability of CYP2E1 expression in humans.
- Does the dose-dependent transition from CYP to GST metabolism represent a biologically plausible mode of action for the nonlinearity of risks associated with DCM?
- The panel agreed that the dose-dependent transition from CYP to GST metabolism represents a highly plausible mode of action. The observed nonlinearity of the mouse tumor response is considered to arise from the nonlinearity of target tissue dose in relation to airborne DCM concentration, rather than from a threshold-limited or other nonlinear relationship between the carcinogenic response and target tissue dose.

Panel/Observer Comments

Uncertainties remain about the detailed mechanisms by which the GST pathway leads to DCM-induced carcinogenicity in mice. Although dibromomethane reacts with ^{6}O-alkylguanine transferase, a DNA repair enzyme, no data specific to DCM are available. Hence it is plausible, but still not known, that DCM inhibits DNA repair in target tissues by interacting with ^{6}O-alkyltransferase. DCM is mutagenic in *Salmonella typhimurium* strains that overexpress GSTT1-1 (Graves *et al.*, 1994; Thier *et al.*, 1993), but it is negative in most other genotoxicity tests. No S-(chloromethyl)glutathione–derived adducts have been identified in animals exposed to DCM, although deoxyguanine adducts have been identified in *in vitro* experiments where DCM was incubated with glutathione, GSTT1-1, and deoxyguanosine (Kayser and Vuilleumier, 2001; Marsch *et al.*, 2001).

It is also known that glutathione depletion results in an apparent increase in the metabolism of DCM to CO in rats, although the mechanism underlying this effect has not been elucidated (Gargas *et al.*, 1986). It has been proposed that glutathione reacts with the intermediate formyl chloride to give S-(formyl)glutathione, which can then be converted to carbon dioxide and formaldehyde. Depletion of glutathione would support the decomposition of formyl chloride to CO. Although

a reaction between formyl chloride and glutathione has not been demonstrated, formyl fluoride does react with thiols to give S-formyl derivatives; hence, this proposal is also plausible.

It was also noted that DNA-protein cross-links have been utilized as an alternative dose metric in studies of the potential effects of GSTT1-1 polymorphisms on estimated cancer risks for humans exposed to DCM (Casanova *et al.*, 1992; El-Masri *et al.*, 1999; Jonsson and Johanson, 2001). Other nonlinear modes of action, such as high-dose effects on cell proliferation in target tissues, especially in the lung, have been suggested, and some panel members urged caution regarding the assumption of a proportional (linear) relationship between tumor incidence and integrated GST pathway metabolites. More data are needed to clarify the potential roles of S-(chloromethyl)-glutathione, S-(formyl)glutathione, and cell proliferation in DCM-induced tumorigenicity.

GSTT1-1 is polymorphic in humans, and the null variant is quite common (Haber *et al.*, 2002). Homozygotic subjects show full capacity for metabolism (high conjugators), whereas heterozygotic subjects show intermediate metabolic capacity (low conjugators), and homozygotic null subjects (nonconjugators) show no GSTT1-1–dependent metabolism. The frequency of nonconjugators varies among the ethnic groups that have been studied (10–64%) and typically ranges from 15 to 25% (Haber *et al.*, 2002). Nonconjugators lack the capacity to bioactivate DCM and should, therefore, be at no increased cancer risk from exposure to DCM. Although GSTT1-1 is involved in the bioactivation of a range of potential carcinogens, no consistent pattern has been observed for a protective effect conferred by the null polymorphism. The issue of potential interindividual differences in CYP2E1 expression levels in humans also needs to be considered in this context, since it is the relative strength of both the CYP and GST pathways in an individual that determines the DCM exposure at which the GST pathway (if one is present) begins to predominate, i.e., at which CYP pathways become saturated.

The presence of GSTT1-1 has also been demonstrated in nuclei isolated from mouse liver, and the intranuclear to cytosolic GSTT1-1 ratio is markedly higher in mouse liver than in human liver (C. R. Elcombe, unpublished studies). Further studies are required to assess the potential implications of these observations for human cancer risk assessment.

PBPK Modeling (Lisa M. Sweeney)

A modification of the original PBPK model for DCM developed by Andersen *et al.* (1987) has been utilized to estimate interindividual differences in the rate of oxidative metabolism of DCM (Sweeney *et al.*, 2004). This model (Fig. 1) was fit to the individual pharmacokinetic data for 13 healthy adult volunteers (10 males, 3 females) exposed to one or more airborne concentrations of DCM (50, 100, 150, or 200 ppm) for 7.5 h/day (4 h in the morning followed by a half-hour break and an additional 3.5 h in the afternoon). Data were obtained for both DCM and CO concentrations in both blood and exhaled breath during exposure and up to 40 h after the end of the exposures. The data are published only in summary form (DiVincenzo and Kaplan, 1981) and have not been used previously in PBPK model development or parameter estimation.

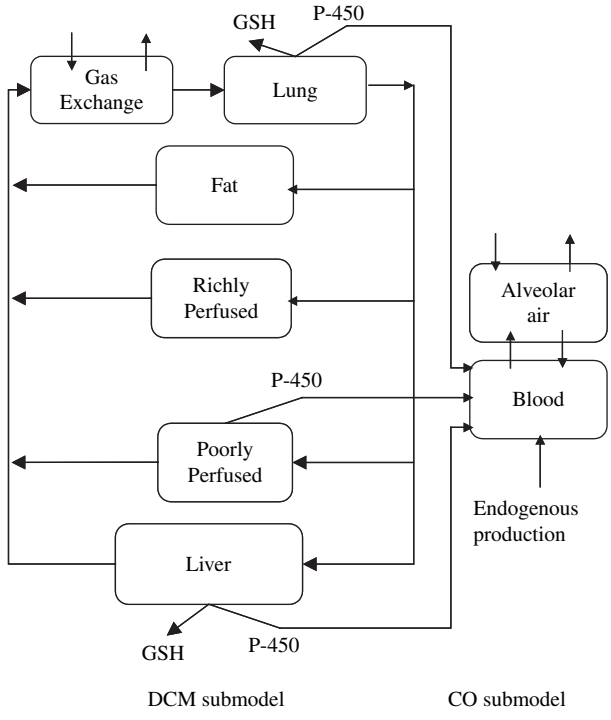

FIG. 1. PBPK Model—Adapted from Sweeney *et al.* (2004).

Efforts to fit the original Andersen *et al.* (1987) model (the "baseline" model) to these data by optimizing only one parameter, the maximal rate of oxidative metabolism in liver (V_{MAXC}), to each individual's blood and exhaled breath time course data yielded unsatisfactory results. Although the Andersen model (with $V_{MAXC} = 6.25$ mg/h/kg$^{0.7}$) generally provided reasonable fits to exhaled breath DCM concentrations during exposure, large discrepancies with the measured exhaled breath DCM concentrations postexposure drove the individually optimized V_{MAXC} parameter to unrealistically high values that ranged from 12 to 5825 mg/h/kg$^{0.7}$. This difficulty was ultimately resolved by adding additional CYP-dependent metabolism (10% of the corresponding liver activity) to the poorly perfused tissue compartment, but it also necessitated the introduction of interindividual variability in the stoichiometric yield of CO. Although the strategy of varying V_{MAXC} and the stoichiometric yield allows flexibility in achieving better time course fits, the estimates of these two parameters are highly correlated. Increasing V_{MAXC} improves the fits to DCM concentrations in blood and exhaled breath, and any overestimation of CO production from a higher V_{MAXC} value can be

compensated for by decreasing the stoichiometric yield of CO from oxidation.

A jackknife optimization procedure that sequentially dropped individual data points from the parameter estimation process was utilized to produce central estimates and confidence intervals for the individual-specific V_{MAXC} values. The approximately three-fold range of V_{MAXC}/K_m values obtained in this manner is consistent with the five individual values previously reported by Jonsson *et al.* (2001).

Charge questions to the panel were the following.

- Are there any other published or unpublished data that should be used in developing the PBPK model?
- The panel discussed other published human data (Jonsson and Johanson, 2001; Jonsson *et al.*, 2001; OSHA, 1997) and concluded that use of these data sets was not likely to materially improve the current PBPK model. The panel agreed that development and estimation of the new model focused appropriately on the extensive individual human data from DiVincenzo and Kaplan (1981) that had not previously been utilized for risk-assessment purposes.
- Is the dose metric related to metabolism via the GST pathway derived from the PBPK model the most appropriate dose metric for human cancer risk assessment?
- The panel considered this dose metric to be the best available at present, but information about the nuclear localization of GSTT1-1 (see previous discussion) may be important in the future. For example, if sufficient data were available, each compartment with GST activity could be split into nuclear and cytosolic subcompartments with the time-integrated GSTT1-1 activity in the nuclear subcompartment serving as the critical internal dose metric for carcinogenicity.
- Is the apportionment of DCM metabolism between the liver and lung implemented correctly and is it consistent with the available information?
- The apportionment between liver and lung was based upon the *in vitro* distribution of enzyme activities and was implemented correctly in the model structure. The ratios of lung to liver CYP and GST activities were taken from the original work of Reitz *et al.* (1988) without modification.

Panel/Observer Comments

The panel discussed the modification made to the baseline model (Andersen *et al.*, 1987) to allow for additional extrahepatic metabolism of DCM. It was recognized that this change in model structure increased whole-body extraction of DCM, which led to a more rapid decline in blood DCM concentrations after the end of the exposure. However, this change also necessitated adjustment of the CO yield parameter downward and separately for each person (resulting parameter range: 1.4- to 4-fold lower than the single value of 0.7 used in the baseline Andersen 1987 model). Some panel members expressed concerns about the impact that varying the stoichiometric yield might have on other aspects of the model but recognized that a mean value of 0.7 for the CO yield parameter (with very tight variance) was used as the prior distribution in the probabilistic risk assessment (discussed in the next section).

There was also a discussion on whether the jackknife procedure for estimating parameter variances had been properly implemented in that, not all data from a given time point (DCM in blood and exhaled breath, CO in blood and exhaled breath) were simultaneously omitted from the model fitting process. This could lead to biased (too tight) estimates of variance and confidence intervals for the individual V_{MAXC}/K_m ratios.

Finally, it was noted that epithelial target cells in the lung airways would be expected to equilibrate with the DCM concentration in inhaled air rather than its concentration in blood. These airway tissues possess both CYP and GST enzymes capable of metabolizing DCM, and the dose-response curves for metabolite production in these tissues would then be shifted to slightly lower inhaled concentrations compared to those for tissues, such as liver, that equilibrate directly with blood. This behavior has been described in detail for styrene (Sarangapani *et al.*, 2002). It may therefore be useful to consider the implications of airway metabolism in relation to GST pathway dosimetry in the lung in DCM-exposed mice and humans.

Probabilistic Risk Estimation (Harvey J. Clewell)

The 1987 cancer risk assessment of the U.S. Environmental Protection Agency (U.S. EPA) for DCM employed deterministic PBPK models for humans and mice that did not allow for interindividual differences in the model's physiologic and metabolic parameters or for any residual (postestimation) uncertainty regarding their true values. Recently, however, Bayesian (MCMC) methods have been used to estimate PBPK model parameters while simultaneously generating quantitative estimates of interindividual variability and residual uncertainty. For example, OSHA's final rule for workplace exposures to DCM was based upon a Bayesian approach to both PBPK model parameter estimation and quantitative risk assessment (OSHA, 1997).

The baseline model for the current work (ENVIRON Health Sciences Institute, 2005) was the original PBPK model (with slight modifications) developed by Andersen *et al.* (1987) to describe DCM uptake, distribution, and disposition in both humans and mice. Following Sweeney *et al.* (2004), extrahepatic metabolism was added to the human version of the model, but it was placed in the rapidly perfused compartment rather than in the slowly perfused compartment. Time course data for blood DCM concentration, exhaled DCM and CO concentrations, closed-chamber air concentration of DCM, and percent carboxyhemoglobin were used in estimating the human model's metabolic parameters. Data from a total of 42 individuals (13 from DiVincenzo and Kaplan, 1981; 12 from Engström and Bjurström, 1977; 14 from Åstrand *et al.*, 1975; and 3 from Stewart *et al.*, 1972) exposed to a range of airborne DCM concentrations both above and below the point of CYP saturation

were employed. Group mean parameter values from Andersen *et al.* (1991) were taken as the prior distribution means.

The mouse version of the model was fit sequentially to time course data from three different types of experiments: closed-chamber DCM inhalation exposures with or without treatment to inhibit CYP2E1 metabolism and intravenous DCM injection. Prior distribution means were taken from Andersen *et al.* (1987), OSHA (1997), and Clewell *et al.* (1993) with prior variances set purposely high, i.e., noninformative, to preclude unnecessary constraints on the model estimation process and to allow the more recent experimental data to have maximal impact on the posterior parameter estimates. The mouse model with posterior mean parameter values was then used to generate point estimates of the internal doses (mg DCM metabolized by the GST pathway/L tissue/d) in the lung and liver resulting from the exposure conditions employed in the NTP mouse bioassay. These internal dose estimates, three-fold to four-fold higher than those used previously by U.S. EPA, were then employed in conjunction with the NTP bioassay tumor data to estimate linearized multistage model parameters and develop central Effective $Dose_{10}$ (ED_{10}) and Lower 95% confidence bound Effective $Dose_{10}$ (LED_{10}) estimates of the internal doses associated with 10% extra risk of lung or liver cancer.

Random Monte Carlo sampling of the human PBPK model posterior parameter distributions (modified to account for the distribution of GSTT1-1 polymorphism phenotypes in the U.S. population) was employed in generating individual human internal lung and liver doses associated with continuous exposure to 1 $\mu g/m^3$. Lung and liver doses from each draw were then multiplied by the lung and liver risk factors ($0.1/LED_{10}$) to generate separate, linearly extrapolated extra risk estimates of lung and liver cancer predicted to arise from this inhalation exposure. Finally the lung and liver risks were added together, and the posterior frequency distributions of predicted liver, lung, and total extra risk were compiled.

The mean combined extra risk was 1.05×10^{-9}, almost 500-fold lower than the current U.S. EPA estimate of 4.7×10^{-7}. However, an interspecies body surface area adjustment employed only in the U.S. EPA computations accounts for 12.6-fold of this large difference, leaving a residual 40-fold difference that can be attributed to various differences between the U.S. EPA and the current PBPK model parameter estimates. The present risk distributions are considered to provide the best estimates developed to date because all available human data sets were utilized to the extent possible in combination with Bayesian estimation methodology that accommodates and quantifies interindividual differences in internal doses in relation to any given airborne DCM concentration.

Charge questions to the panel were the following.

- Are the major assumptions used in the probabilistic risk determination adequately described and justified?

- The panel considered the major assumptions to be generally appropriate and adequately described in the report (ENVIRON Health Sciences Institute, 2005). Inclusion of more technical details would, however, allow for better understanding of the MCMC estimation process. For example, the rationale for the selected prior distributions could be described in more detail, as could the sensitivity of the final results to the assumed prior distributions. In addition, it would be helpful to describe more fully and justify the MCMC convergence criteria (see additional comments below).

- Is the procedure used for parameter estimation reasonable?

- The panel believed that appropriate procedures were used for parameter estimation. All available human data were used. Prior values were selected from multiple sources (Andersen *et al.*, 1987, 1991; Bois, 2000; EPA, 1991; OSHA, 1997) that reflected, in each case, the source considered to represent the best scientific evidence for a parameter. Some physiological and metabolic parameters were constrained to reflect biological plausibility, although this criterion was not imposed on all PBPK model parameters.

- Is the MCMC analysis for DCM an appropriate approach for characterizing the uncertainty in PBPK model output?

- It is appropriate, although there may be alternative ways to utilize the animal data more fully in assessing uncertainty and variability. The present approach was aimed primarily at characterizing the impact on estimated risks of interindividual variability of critical pharmacokinetic parameters in humans as inferred from the DiVincenzo and Kaplan (1981) and other available human time course data, as well as the additional interindividual variability attributable to the GSTT1-1 genetic polymorphism. Other uncertainties, such as structural PBPK model uncertainty and uncertainty in the critical metabolic parameters for the mouse, were not incorporated in the present MCMC-based risk estimation.

- Is the incorporation of GSTT1-1 genetic polymorphism in development of a unit risk factor appropriate for DCM?

- The panel agreed that incorporation of this polymorphism into a population-based unit risk distribution appears to have been implemented appropriately.

- Was the statistical methodology used appropriately in the risk estimation?

- The approaches and conclusions appear to be appropriate, although the panel suggested some improvements (see specific recommendations below). In particular, more technical detail would be helpful in understanding the sources of the 40-fold difference between the earlier U.S. EPA and present unit risk estimates. Overall, the panel concluded that the current results (ENVIRON Health Sciences Institute, 2005) reflected the best available data and science for DCM risk assessment.

Panel/Observer Comments

The panel concluded that the probabilistic risk assessment had been well conducted overall, but suggested several areas where more information could be provided to improve understanding of the analysis. More detail on characteristics of

the Markov chains would be valuable, including why different chain lengths were used for different data sets (treated mice, humans, etc.). The burn-in period of the chains and the thinning interval used in developing the posterior distributions of model parameters should also be discussed. A default thinning interval of five was assumed without apparent justification. Bayesian modelers typically use a thinning interval that leads to negligible autocorrelation in the thinned chains.

It would also be useful to describe explicitly how variability and uncertainty were addressed within the context of the probabilistic analysis. Variability is inherent in parameters that represent measured traits in a population, e.g., body weights or metabolic rates in a group of individuals. Uncertainty reflects residual ignorance about the true population parameters, e.g., the true mean and variance of the V_{MAX} distribution are unknowns. The discussion of the hierarchical model design should address these issues in greater detail. For example, at each step of the Markov chain, parameter values for all individuals were drawn from the same sampling distribution, but the parameters of this sampling distribution also have distributions that reflect residual uncertainty regarding the true population parameter values. More details related to how individually fitted parameter values were obtained should also be provided. These can be inferred from the discussion, but explicit specification, perhaps as an appendix, would be helpful. It would also be helpful to include a table summarizing the prior and posterior distribution means and variances of all model parameters.

It would also be desirable to incorporate all available human data in the estimation process, including, if possible, group mean data for which individual values are not available. If the exclusion of the group mean data was a result of software restrictions, then there may be a work-around. For example, if only means and variances were available for the group data, then pseudodata could be generated by random sampling from distributions with these means and variances. This pseudodata could then be generated multiple times to check the sensitivity of the model fitting process to this imputation.

PBPK modeling is appropriate for generating a dose metric that is believed to be more closely correlated with the adverse response of interest than is administered dose. Such modeling will not, however, necessarily reduce uncertainty. It may simply reflect more accurately our limited state of knowledge. It is worth noting that a major source of uncertainty is the form of the PBPK model itself (structural uncertainty). Distinct tissues are pooled into single compartments because these tissues are expected to behave in a similar manner. The version of the model published by Sweeney *et al.* (2004) locates extrahepatic metabolism in the poorly perfused compartment (Fig. 1), whereas the version described in this presentation (ENVIRON Health Sciences Institute, 2005) assigns it to the richly perfused compartment. It was recognized that the main contribution of additional extrahepatic metabolism in either of these compartments is increased whole-body extraction of DCM, leading to improved fits to blood DCM levels post-exposure, and that the precise localization of the extrahepatic component is not likely to have a major impact on the final dosimetry estimates. There is, nonetheless, a desire for consistency between the updated model structures.

Consideration should also be given to incorporating extrahepatic CYP metabolism into the mouse model used for cancer risk estimation in order to be consistent with the human model structure. Although variability and uncertainty are incorporated into the exposure and internal dosimetry component of the risk-assessment computations (the human PBPK model), variability of dosimetry in the risk estimation component (the mouse PBPK model coupled with the linearized multistage model) is not considered. A single LED_{10} value representing only the binomial sampling variability of the tumor endpoints is the only output from this potency estimation component. It would, therefore, also seem important to consider including the uncertainty in the mouse PBPK model parameters along with the sampling variability of the mouse tumor responses in a more comprehensive, fully probabilistic risk assessment. It would also be useful to know how much the potency estimates from other dose-response models would differ from the reported linearized multistage model estimate.

Some Recent Epidemiologic Study Findings (Karl E. Wende)

Both case-control and retrospective cohort mortality studies of DCM were reviewed. Case-control studies of astrocytic brain cancer (Heineman *et al.*, 1994) and breast cancer (Cantor *et al.*, 1995) have suggested associations with DCM exposure. The former study of 300 cases and 320 controls employed next-of-kin interviews to ascertain work histories from which the likelihood and intensity of exposure for six industrial solvents, including DCM, were constructed based on jobs classified by the Standard Industry Classification and Standard Occupational Classification codes. The authors reported significantly elevated odds ratio trends with increasing probability of DCM exposure among those with high cumulative exposure scores or at least 21 years of employment in exposed jobs. The latter study included over 33,000 women with the cases identified through death certificates. Usual occupations and industries were also obtained from the death certificates, and likelihood and intensity of workplace exposures were estimated using a job-exposure matrix. When the analyses were adjusted for age and socioeconomic status, the authors found modestly elevated odds ratios among white females in the middle exposure probability category, and among black females in the highest exposure level category. Both studies used imprecise proxy measures of DCM exposure.

The cohort mortality studies include two cohorts of Eastman Kodak Company workers (Hearne and Pifer, 1999) and a cohort of British Imperial Chemical Industries workers (Tomenson *et al.*, 1997), all involving DCM exposures arising in photographic film base manufacturing. In addition, the mortality of

workers at two textile fiber manufacturing plants where DCM was employed as a solvent has been studied (Hoechst-Celanese, Rock Hill, SC [Lanes *et al.*, 1993], and Cumberland, MD plants [Gibbs, 1992; Gibbs *et al.*, 1996]).

As a group, the cohort mortality studies showed uniformly and significantly lower than expected mortality from all causes, all malignant neoplasms, and ischemic heart disease. For the specific sites corresponding to those that were elevated in the NTP rodent bioassays (lung and liver in the mouse, mammary gland in the rat), the risk of death from trachea, bronchus, and lung cancer was also significantly below expectation, whereas liver cancer mortality was below expectation in all except one textile cohort, where it was nonsignificantly elevated (4 deaths vs. 1.3 expected). Breast cancer mortality was below expectation in the only cohorts with substantial numbers of female workers (Lanes *et al.*, 1993).

Several other sites (pancreas, cervix, prostate, brain, and other central nervous system, and leukemia) were only inconsistently and nonsignificantly elevated in some of the cohorts or cohort subgroups, and no coherent pattern of excesses was apparent across the cohort studies. A statistically significant trend for leukemia mortality in relation to cumulative DCM exposure was noted in the Kodak (1964–1970) cohort (Hearne and Pifer, 1999), but this was based on only six total deaths of three with these deaths observed in the highest cumulative exposure category. Interestingly, the highest exposure subcohort in the study of textile workers showed a reduced risk of leukemia mortality (Gibbs, 1992).

Charge questions to the panel were:

• Are outcomes observed in the epidemiological studies consistent with those noted in the animal toxicity tests?

• The panel agreed that there is no clear evidence of increases in the major sites (liver and lung) observed in the animal studies. Although the breast cancer findings in the Cantor *et al.* (1995) study are consistent with increased mammary tumor incidence in DCM-exposed female rats, the significant limitations in the identification of exposures in both case-control studies need to be carefully considered in weighing the results. These studies are far more useful in hypothesis generation than in confirmation of elevated risks. Nonsignificant increases in some cancers have not been consistent across studies. Furthermore, no meta-analyses of data from the four occupational cohorts have been conducted.

• Are dose-response analyses of observations in the epidemiological studies consistent with the dose responses for outcomes noted in the laboratory animal studies?

• The panel agreed that there were no consistent findings in the epidemiology studies and that none would be expected based upon quantitative risk extrapolations from the animal study findings. While the increase in brain cancer incidence reported in the Heineman *et al.* (1994) case-control study suggested a dose response, DCM exposure was not adequately characterized. No human excesses or dose responses have been reported for cancers of the primary animal target tissues (liver and lung). However, no appropriate internal analyses of dose responses have been reported for many outcomes in the cohort studies.

• Do the epidemiological studies related to both metabolism and health effects provide sufficient evidence to be consistent with results from PBPK modeling studies?

• Additional information on tissue-specific GSTT1-1 and CYP2E1 activities may be helpful in assessing adequately the epidemiologic study findings and expectations based upon PBPK modeling.

• Do the few observations of increased cancer mortality reported in epidemiological studies at sites not identified as DCM targets in laboratory animal studies have sufficient biological plausibility and consistency to be considered serious risks?

• The reported elevations are not consistent across the human studies. Only sporadic low-level responses have been reported for brain, leukemia, and pancreatic cancer. The apparent dose-response trend for leukemia mortality in the Kodak (1964–1970) cohort is based on very small numbers.

Other Panel/Observer Comments

Although the few sites with slightly elevated cancer mortality in the epidemiological studies are not consistent with the target sites observed in the animal carcinogenicity studies, humans do not always manifest the same cancers as are seen in animal species. Concordance across species need not be one-to-one. If the exact mechanism of cancer formation in DCM-exposed mice were known, we might be better able to determine what sites to expect to be elevated in DCM-exposed humans.

Airborne DCM concentrations in the epidemiologic studies of DMC-exposed workers are far lower than those that have caused cancer in mice. Therefore, humans have not been exposed to comparable biologically effective doses, based on the known quantitative differences in metabolism across species. An important issue for DCM is the continued improvement in specification of critical target tissue doses in both animals and humans.

In general, the epidemiologic studies provide little evidence, either positive or negative, about coherence with the predictions of the PBPK and probabilistic risk-assessment models. The case-control studies have serious flaws, including the utilization of deceased subjects and indirect (and imperfect) methods for determining the potential for DCM exposure in cases and controls. The cohort studies are small with little power to detect excess risks of rare cancers, such as human liver cancer. It may be useful to compute power retrospectively for the various sites that have been reported in both animals and humans. A meta-analysis of the cohorts with, or even without, pooling of data would be an important step toward increasing the overall power of the cohort studies and increasing the sensitivity of

dose-response assessments for any cancer sites of interest. Concordance of target sites across studies, biologic plausibility, and dose-response relationships consistent with expectations based on PBPK modeling should be demonstrated before weak increases at multiple different sites are considered as plausible evidence for a carcinogenic effect of DCM in humans.

WORKSHOP SUMMARY

The panel was asked by panelist Dr. David Gaylor to express an opinion about the differences in risk noted in this assessment compared to the previous U.S. EPA (1991) assessment. The summary statement below was discussed by the panel without disagreement.

In general, the panel noted that this assessment used new human data in developing a probabilistic model for assessing potential human cancer risks and has introduced consideration of GSTT1-1 polymorphisms in the assessment. This model is well supported by mode-of-action studies conducted over the past decade, and it employed a PBPK model that is well validated through previous applications with animal and human pharmacokinetic data and extended with the individual subject data from the DiVincenzo and Kaplan (1981) studies. The small differences in parameterization between the Sweeney *et al.* (2004) PBPK model and the Clewell model for quantitative risk assessment (ENVIRON Health Sciences Institute, 2005) do not appear unreasonable, and both provide good fits to the available time course data. The probabilistic assessment was adequately conducted using appropriate statistical tools. The resulting reduction in estimated risks, some 40-fold (in addition to the 12.6-fold reduction due to elimination of the interspecies body surface area adjustment factor), may not be an exact representation of the true risk differences, but it nonetheless argues for much lower risks than those indicated in the most recent U.S. EPA (1991) assessment. The panel agreed that the current state of the science for DCM should lead to substantial reductions in the potential human cancer risks that might reasonably be expected from DCM exposure.

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