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Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride): Pharmacokinetics, Mechanism of Action, and Epidemiology

Review Draft

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UPDATE TO THE HEALTH ASSESSMENT DOCUMENT AND ADDENDUM FOR DICHLOROMETHANE (METHYLENE CHLORIDE): PHARMACOKINETICS, MECHANISM OF ACTION, AND EPIDEMIOLOGY

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Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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FOREWORD

This document is the result of a yearlong intensive review and evaluation of the latest data that have a bearing on risk assessment for dichloromethane (DCM; methylene chloride). These data, and the most current methods for their analysis, have been reviewed in an effort to improve the quantitative risk estimates for DCM. In addition, I hope that this review and the attempt at applying state-of-the-art methodologies will help to advance the science of risk assessment.

Several key issues have arisen as a result of the new data that have been provided to the federal regulatory agencies. The most notable is the impact of pharmacokinetic analysis on risk extrapolation. Physiologically based pharmacokinetic models are used to estimate organ-level concentrations of DCM and its metabolites. Such organ-level concentrations can serve as measures of the delivered dose. This type of pharmacokinetic analysis raises two important questions. First, can sufficiently reliable estimates of delivered dose be made so that they constitute an improvement over the use of applied dose as a basis for risk extrapolation? Second, how are such delivered-dose estimates to be used in the quantitative extrapolation of risk from experimental animals to humans exposed to much lower doses?

Two possible applications of how pharmacokinetic information and data may be incorporated into the quantitative risk assessment are developed. The first (Method 1) incorporates interspecies

differences in pharmacokinetics while the second (Method 2) only incorporates differences resulting from high- to low-dose extrapolation. The major uncertainties associated with each approach are discussed. For the present, using pharmacokinetic data and models for interspecies extrapolation results in a reduction of the estimated risk from the applied-dose estimate by almost ninefold. Similarly, using the pharmacokinetic models for high- to low-dose extrapolation only, the risk would be reduced from the applied-dose estimates slightly more than twofold. The two methods differ in the assumptions that are made and are not equally sensitive to one of the key metabolic rate constants.

For the present, EPA scientists have used Method 1, which results in an ninefold reduction from previous unit risk estimates based on applied dose. CPSC scientists, on the other hand, have used Method 2 which results in an approximate twofold reduction. OHSA and FDA scientists are in the process of deciding what, if any, modifications to make to present risk numbers.

It would be unwise to read too much importance or significance into changes in the unit risk of a few fold when pharmacokinetic data are used in either Method 1 or Method 2. This document outlines uncertainties in the structure and parameter values of the pharmacokinetic models. Although it is difficult to define these uncertainties in quantitative terms, it is clear that model projections of internal doses could vary, perhaps up to several fold, without contradicting currently available model validation data. Moreover, there are many

uncertainties as to the biological effects of those internal doses that overshadow any error in their estimation. Species differences in responsiveness--and within-species differences in susceptibility of various tissues--are unclear. Perhaps the largest uncertainty lies in the question of the relative carcinogenicity of high and low doses, owing to the lack of knowledge about the mechanism of DCM's carcinogenic action.

Rather than focusing on exactly how much the risk extrapolation has been changed by the use of pharmacokinetic information, it is instructive to examine how little it has been changed. Perhaps the most important result of the these analyses is that, in the case of DCM, pharmacokinetic considerations have not revealed a great error inherent in using applied dose as a surrogate for internal or delivered dose.

Thus, Method 1 has been chosen not because it is felt to be the only valid approach (it is not yet clear which approach, if any, can be used with confidence), but rather because it represents the best use of the available information in a manner closely consistent with what EPA has done with other chemicals, such as tetrachloroethylene and trichloroethylene. In view of the uncertainties involved, the changes in DCM's carcinogenic potency that result from different uses of the available pharmacokinetic information are not, in practical terms, very different. Discussion of the issues has been worthwhile because of their theoretical importance rather than their practical significance in the present case. For other compounds (or for DCM itself, upon

itself, upon the introduction of new data), the distinction among extrapolation methods may have much greater practical consequences and the question of choice among methods will have to be re-examined. EPA staff will then be required to look for data and ways with which to reduce the uncertainties around a given estimate. I hope that the peer review process that this document will undergo will also help in the quest for this knowledge.

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PREFACE

This document was prepared by staff of the Office of Health and Environmental Assessment (OHEA) and the Office of Toxic Substances (OTS) of the U.S. Environmental Protection Agency (EPA) as part of the Integrated Chlorinated Solvents Project. It represents the latest technical review and assessment of dichloromethane (DCM). The document is not intended to replace the Health Assessment Document (HAD) and Addendum for Dichloromethane but to update them by providing an evaluation of data and risk assessment methodologies that have become available since their publication. The update comprises two documents: one prepared by EPA and a second prepared by the Health/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project.

This EPA document represents EPA's analysis of the weight of evidence regarding DCM's carcinogenic potential for humans; it includes revised cancer risk estimates which take into account the new information submitted to date on pharmacokinetics, mechanism of action, and epidemiology, and discusses in some detail methods for incorporating pharmacokinetic information into the cancer risk assessment. The EPA document draws on the body of work developed by the HRAC, an interagency committee that was established to evaluate the health effects caused by DCM and five other halogenated solvents. As part of its work on halogenated solvent compounds, the HRAC reviewed and evaluated all of the information

recently submitted to EPA and other federal agencies on DCM's potential to cause cancer and other toxic effects.

The HRAC analyses, which comprise a second document titled "Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments," deal with several aspects of the risk assessment.

ABSTRACT

This document represents EPA's analysis of the weight of evidence regarding the carcinogenic potential of dichloromethane (DCM, methylene chloride) for humans. It includes revised cancer risk estimates that take into account the newest information on pharmacokinetics, mechanism of action, and epidemiology, and it discusses, in some detail, methods for incorporating pharmacokinetic information into the cancer risk assessment. This document draws on the body of work developed by the Hazard/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project, an interagency workgroup. For the present, EPA scientists have used methods that result in an approximate ninefold reduction from previous unit risk estimates which were based on applied dose.

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This document represents the joint efforts of the staff of the Office of Health and Environmental Assessment (OHEA) and the Office of Toxic Substances (OTS) of the U.S. Environmental Protection Agency (EPA).

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1. INTRODUCTION

Dichloromethane (DCM, methylene chloride) is a multipurpose solvent with application in paint stripping, metal cleaning, foam blowing, electronics, and chemical processing. It is also a component in certain aerosol propellant mixtures. Because of its many applications, a large number of people may be exposed to DCM in the workplace, through use of consumer products, or from emissions to ambient air.

Animal bioassays published in the 1980s raised concern that DCM could cause cancer in humans. Several bioassays reported an elevation in tumor incidences in mice and rats exposed to this chlorinated solvent. In response to these bioassay data and concerns about other potential chronic effects, EPA's Office of Air and Radiation (OAR) requested that the Office of Health and Environmental Assessment (OHEA) prepare a Health Assessment Document (HAD) on DCM. The HAD, which reviewed data relevant to acute and chronic effects, was published in February 1985.

New bioassays published in 1985 by the National Toxicology Program (NTP) intensified concern for DCM's potential to cause cancer. EPA's Office of Toxic Substances (OTS) began a regulatory review to determine whether the risks posed by any or all uses of DCM were sufficient to warrant priority consideration for regulatory action under section 4(f) of the Toxic Substances Control Act (TSCA). OAR, already involved in investigating

whether ambient sources of DCM should be regulated under the National Emissions Standards for Hazardous Air Pollutants (section 112) provision of the Clean Air Act (CAA), requested that OHEA prepare an addendum to the HAD to evaluate the new data from the NTP.

In May 1985, OTS published (U.S. EPA, 1985c) a finding under TSCA section 4(f) that DCM may present a significant risk to humans of serious and widespread harm from cancer. The new NTP bioassays provided sufficient evidence to upgrade DCM from a possible to a probable human carcinogen and led to a positive 4(f) finding. Following the 4(f) action, OTS and OAR combined efforts and a single coordinated assessment was published by OHEA in July 1985 as an Addendum to the HAD for DCM. In the Addendum, EPA classified DCM as a probable human carcinogen (Group B2) as defined by the EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). The assessment led to publication of an EPA Advance Notice of Proposed Rulemaking in October 1985 (U.S. EPA, 1985d).

EPA was not alone in responding to the NTP's new bioassay data. Following release of the NTP data, several other federal regulatory agencies began to investigate the health effects posed by DCM. The Consumer Product Safety Commission (CPSC) released a briefing package in June 1985 announcing the staff's intent to pursue voluntary labeling actions. Subsequently, CPSC began a rulemaking procedure to determine whether or not DCM should be designated a hazardous substance, and published a Federal

Register notice (U.S. CPSC, 1986) requesting comment on the proposed rule. In November 1985, the Food and Drug Administration (FDA, 1985) proposed a ban on the use of DCM in hairsprays. The Occupational Safety and Health Administration (OSHA) began an investigation into the risks associated with occupational exposures to DCM, and published an Advance Notice of Proposed Rulemaking in November 1986 (OSHA, 1986).

The multiple assessments called for a coordinated effort on the part of the various agencies to ensure consistency in regulatory decision-making. To that end, EPA proposed developing a common integrated assessment covering not only DCM, but other chlorinated solvents that could serve as substitute chemicals as well. The agencies developed an integrated strategy to assess the major occupational, ambient, and consumer sources of exposure and risk from DCM, tetrachloroethylene, trichloroethylene, carbon tetrachloride, methyl chloroform, and CFC-113.

The integrated strategy led to the establishment of a committee composed of representatives from EPA, CPSC, OSHA, and FDA to assess data relevant to the health risks that might be associated with exposure to the chlorinated solvents. The committee, called the Health/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvent Project, was charged with reviewing and updating, if necessary, the existing hazard/risk assessments on the six solvents. Separate committees were set up to resolve issues concerning ambient air, occupational and consumer exposure, and to address economic issues through the

development of a risk/benefit analysis.

In response to the NTP bioassay and the federal regulatory agencies' investigations, industry and other external groups developed new data on DCM, focusing primarily on species differences in metabolism and the mechanism of tumor initiation. The HRAC has made it a top priority to evaluate these data, acknowledging the need for review of EPA's final risk assessment by the Science Advisory Board, rulemaking procedures underway by CPSC (1986), OSHA (1986), and FDA (1985), and the responsibility of addressing public comments. The HRAC's evaluations of the new data serve as the basis for EPA's updated risk assessment of DCM.

2. OVERVIEW OF DICHLOROMETHANE CANCER HAZARD/RISK ISSUES

In May of 1985, EPA's Office of Toxic Substances found that DCM met the criteria for priority review under section 4(f) of the Toxic Substances Control Act (TSCA). Underpinning the 4(f) decision was the conclusion that DCM should be considered a probable human carcinogen, as defined by EPA's Guidelines for Carcinogen Risk Assessment.

In assessing the cancer hazard posed by exposure to DCM, primary consideration was given to the evidence of carcinogenicity from the NTP's animal studies (1985). The NTP carcinogenesis bioassays clearly demonstrate that DCM is oncogenic in two species of laboratory animals, rats and mice, exposed at different dose levels via the primary route of human exposure to DCM, inhalation.

In the mouse bioassay, DCM induced a dose-dependent, statistically significant increase in liver and lung adenomas and carcinomas in male and female mice exposed through inhalation for a lifetime at concentrations of 2000 or 4000 ppm. Tumor incidences were as follows: at 2000 ppm, 30/48 female mice and 27/50 male mice developed lung tumors; 16/48 female mice and 24/49 male mice developed liver tumors. At 4000 ppm, 41/48 female mice and 40/50 male mice developed lung tumors; 40/48 female mice and 33/49 male mice developed liver tumors.

In the rat bioassay, DCM induced a statistically significant increase in benign mammary gland tumors, of a type not expected

to progress to malignant tumors (McConnell et al., 1986), at the two highest doses in female rats exposed at 1000, 2000, or 4000 ppm. Male rats developed mammary gland fibroadenomas at 4000 ppm, but only at a marginally significant rate. The NTP interpreted their study as showing clear evidence of animal carcinogenicity, and data from the NTP bioassay on mice are the basis of the regulatory agencies' estimates of human risks at expected human exposures.

A study of Syrian golden hamsters exposed to DCM at inhaled doses of 500 to 3500 ppm was negative, but several chronic studies of mice and rats, including inhalation studies by Dow Chemical Company (1980, 1982) and a drinking water study by the National Coffee Association (NCA) (1982 a, b; 1983), reported an increase in tumors in rats and mice at sites corresponding to the sites observed in the NTP bioassay. One of the Dow studies (1980) (inhalation at 1500 to 3500 ppm) reported an increase in salivary gland sarcomas in male rats. These tumors have not been repeated in other studies. Results of the Dow and NCA studies, conducted at doses below those used in the NTP bioassays, were not statistically significant, with the exception of the salivary gland tumors in male rats.

Based on an estimated risk comparison with the NTP bioassay data, EPA concluded that despite the lack of statistical significance, the results of the Dow and NCA studies were not clearly inconsistent with those of the NTP bioassays. For example, comparing the NCA and NTP unit risk numbers (estimated

using the multistage model) for liver tumors in male mice, the 95% upper confidence limit (UCL) for the NCA study was estimated to be 0.78×10^{-3}; the UCL derived from data on male mice in the NTP study was 0.195×10^{-3} (U.S. EPA, 1985b).

At the time of the 4(f) decision, data on humans exposed to DCM in the workplace were considered to be inadequate for judging carcinogenic potential. Data from two epidemiologic studies did not show evidence of a significant increase in deaths from lung or liver cancer in exposed workers, but these studies had insufficient statistical power to detect increased risks as predicted using the upper-bound estimate derived from the NTP bioassay on mice.

Based on the evidence, EPA concluded that DCM should be classified as a probable human carcinogen, group B2. This classification signifies that evidence of animal carcinogenicity as provided by the NTP bioassays is sufficient, but data from human studies are inadequate. CPSC, FDA, and OSHA, after reviewing the DCM database, came to similar conclusions.

In response to EPA's 4(f) announcement in 1985 and the initiation of investigations by CPSC, OSHA, and FDA, a number of comments and studies were submitted to the federal agencies advancing reasons why the results of the NTP bioassay on DCM in rats and mice should not lead to the conclusion that DCM presents a high risk to humans. The major criticisms of the preliminary assessments suggest that (1) current DCM risk estimates overestimate risks to humans because they ignore species

differences in metabolism and pharmacokinetics; or (2) the carcinogenic response shown by mice is unique to that species, i.e., the mechanism by which DCM causes cancer in mice is not expected in humans.

Addressing these criticisms calls for a brief review of DCM metabolism. DCM is metabolized in mice, the species which showed a clear carcinogenic response, by two routes; one mediated by the cytochrome P-450 oxidative system [often referred to as the mixed function oxidase (MFO) pathway], and the other by the glutathione-S-transferase system (also known as the GST pathway). Both pathways may be active in mice at low doses, but at higher doses the MFO pathway becomes saturated and the metabolic load is increasingly shifted to the alternative GST pathway. Recent studies (CEFIC, 1986e) indicate that the GST pathway is less active in rats, hamsters, and humans than in mice.

Arguments against the conclusion that DCM presents a risk to humans take the position, in general, that the carcinogenicity of DCM is due to reactive metabolites produced by the GST metabolic pathway, and that this pathway is significantly active only following saturation of the MFO pathway, i.e., only at high doses. Further, the GST pathway is assumed to be the sole carcinogenic pathway and to be far less active in humans than in mice, the test species in which malignant tumors have been observed. Finally, some hypothesize that the metabolites of the GST pathway are not reactive with DNA, but initiate cancer in mice through some alternative mechanism such as specialized cell

toxicity or increased cell turnover, events unlikely to occur at low doses and possibly irrelevant to humans. One might conclude from these assumptions that the human risk for developing tumors from exposure to DCM is very low, that it may not exist below some threshold level, or that there may be no risk to humans whatsoever.

3. APPLIED-DOSE RISK ASSESSMENT

The position that DCM presents a low risk to humans is in contrast to the federal regulatory agencies' earlier risk assessments, which indicate that risks to humans at expected human exposure levels may be high. The agencies' initial assessments are based on the applied-dose multistage model procedure for estimating risk (excepting FDA's assessment which is based on exposure concentration and a linear extrapolation procedure similar in principle to the underlying principle of the multistage model), and range from 2.3×10^{-6} per ug/m^3 (CPSC) to 4.1×10^{-6} per ug/m^3 (EPA). This range reflects relatively small differences in assumptions concerning such factors as scaling between species and the use of upper confidence limits (UCL) versus maximum likelihood estimates (MLE). The major assumptions of these assessments, including FDA's, are the same, however.

The applied-dose procedure as used by the federal agencies assumes that risks are a function of the dose taken into the body. It treats the body as a whole unit in which cancer may potentially arise sometime after exposure to DCM. Further, it assumes that the general similarity of mammalian anatomy, physiology, and biochemistry justifies extrapolation from rodents to humans. It incorporates the concept, known as low-dose linearity, that the carcinogenic response seen at low doses would be directly proportional to low-dose exposure.

The applied-dose procedure has several drawbacks: it does

not account for certain metabolic differences between species; it does not account for differences in metabolic reactions at high and low doses; and it does not differentiate between the parent compound and its metabolite(s) or combinations of these, as the carcinogenic species. These shortcomings mean that the procedure does not consider any nonlinearities that may exist between the dose taken into the body (applied dose) versus the dose that actually reaches the target tissues (internal dose), and it does not estimate risk based on the level of a specific carcinogenic species, i.e., the level of a particular metabolite, for example. Risks estimated by this method may, therefore, be over- or under-estimates.

Nevertheless, when definitive knowledge of species-specific biological traits that might affect carcinogenicity, of high- to low-dose differences in metabolism, or of the carcinogenic species are lacking, extrapolating risks to humans using applied dose and the multistage model provides a reasonable approach for dealing with these kinds of uncertainties. This procedure may have some empirical support. For about two dozen chemicals, carcinogenic potency can be estimated directly from available epidemiology and these estimates agree, in general, with projections based on applied-dose extrapolation from animal data [Allen et al. (1986) analyzed 23 substances including industrial chemicals, drugs, food additives, and tobacco smoke].

4. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Some of the uncertainties associated with the applied-dose method may be reduced through information provided by physiologically-based pharmacokinetic models. Such models describe the organ level disposition of a chemical and its metabolites as they vary over time and dose. Given the proper parameters describing blood flows, metabolic rates, and exchanges of compound between tissues, blood, and air, well-validated pharmacokinetic models should be able to account for any non-linearities in the level of target-organ dose of the parent compound or any of its metabolites that may occur with increasing or decreasing applied dose. Further, by adjusting these parameters to reflect species differences, pharmacokinetic models may answer some questions concerning interspecies differences in chemical disposition.

Before applying pharmacokinetic models to risk assessment, however, one should have a clear idea of how such models can reduce the uncertainties inherent in risk extrapolation using the applied-dose procedure. A pharmacokinetic model can account for differences in the disposition and metabolism of a chemical in the body as they vary from dose to dose. Thus, it may be quite useful in reducing the uncertainties associated with extrapolating from high- to low-dose exposures. Questions, such as differences in sensitivities of target-tissues (both within

species and between species) to concentrations of chemicals, whether the site of carcinogenic response would be the same from one species to the next (site concordance), and which chemical is actually the carcinogenic species, cannot be answered by pharmacokinetics. The extrapolation of carcinogenic potency across species depends on many factors in addition to pharmacokinetic differences. Even when pharmacokinetic models are well validated as to their ability to determine internal doses, questions remain as to how this information should be used to alter the extrapolation of risk from rodents to humans. In the sections that follow methods of developing and using internal dose information to assess risks associated with exposure to DCM are discussed.

Two physiologically based pharmacokinetic models have been formulated to predict the disposition of DCM and its metabolites in the body. The model developed by Andersen et al. (1986, 1987) is based on inhalation exposure to DCM and is essentially a modified version of an earlier model developed to describe the disposition of styrene (Ramsey and Andersen, 1984). Andersen and coworkers have stated that if the results of their model were taken into account in estimating risk, risk estimations would be greatly reduced from those published in the EPA Addendum to the HAD for DCM (U.S. EPA, 1985b) and documents from CPSC, OSHA, and FDA.

Angelo et al. (1984) developed a physiologically based pharmacokinetic model describing the disposition of DCM following

exposure by intravenous dosing and by gavage. The HRAC's review (1987) of this model and a series of papers (Angelo et al., 1986a, b; Angelo and Pritchard, 1984) revealed the model used by Andersen and Reitz and the model used by Angelo et al. to be quite different in structure. The existence of two pharmacokinetic models for DCM, differing markedly in structure, demonstrates that the appropriate way to mathematically model such a complex biological process is not self-evident. The model used by Andersen and Reitz, however, is based on inhalation exposure, the route of exposure most important to human risk, and its results are more directly applicable to the federal regulatory agencies' estimates of risk than are those from the model used by Angelo et al. The HRAC (1987) studied the model used by Angelo et al. chiefly for the insights it provides concerning the model used by Andersen and Reitz.

The model used by Andersen and Reitz is designed to estimate the tissue-level doses of DCM (or a metabolite) that result from any given animal or human exposure. Confidence in the model's ability to improve risk estimation depends, however, upon the degree to which the model can be shown to be valid. The HRAC (1987) thoroughly evaluated the model used by Andersen and Reitz and found it to provide a plausible description of the uptake and distribution of DCM. Development of such a model offers great promise for improvement in the accuracy of estimating target-tissue concentrations. At present, however, certain components of the model are not well validated, a matter which

must be considered when determining the degree of confidence that currently can be placed in the model's results.

4.1. MODEL STRUCTURE

Three points about the structure of the model should be noted but are considered to be of minor importance. First, Andersen et al. (1986, 1987) chose to model the lung as a two-compartment region. One compartment acts as the gas exchange region between blood and air and the other as the metabolic region for the metabolism of DCM. Although such a model of the lung is less than a true physiologic representation, it introduces no obvious source of error.

Second, the GST pathway is represented in the model by a single rate constant; Andersen et al. assumed that the rate of metabolism by this pathway will be directly proportional to DCM concentration, even at high exposures. In contrast, data from the European Council of Chemical Manufacturers' Federation (CEFIC, 1986f) indicate the rate of GST metabolism to be saturable at very high substrate concentrations. The CEFIC rate is described by Michaelis-Menton kinetics, which requires the estimation of two metabolic parameters. The two representations of GST metabolism are essentially equivalent in the range of substrate concentrations that the model is concerned with, however.

A third structural element may have more significance. The model does not account for compartmentalization of DCM within the tissues of the individual organs. Data from Angelo et al. (1984)

indicate that under some conditions of exposure, DCM may sequester into the lipid rich regions of the various organs. Compartmentalization may affect the rate of disposition of DCM or its metabolites in the body's tissues; thus, failure to take compartmentalization into account may lead to errors in the estimation of DCM metabolism over time and exposure.

4.2. MODEL INPUT

4.2.1. Partition Coefficients

Andersen et al. (1986, 1987) determined tissue/air partition coefficients in vitro by observing the equilibrium distribution of DCM between homogenized tissue and air. This method does not determine these coefficients for intact tissue; it may, therefore, introduce error into the values of the partition coefficients which, in the model, represent intact tissues. As might be considered a priori, other studies have shown that using homogenized tissues does not account for all the physical and physiologic processes going on in vivo that may affect the partitioning.

Further, the blood/air partition coefficients, which Andersen et al. measured, reflect unexpectedly large differences between the coefficients for mice and rats. CEFIC (1987b) indicated that their measurements of preliminary blood/fat partition coefficients do not repeat Andersen et al.'s findings. The final results of the CEFIC work on blood/fat partition coefficients are expected to be available in the summer of 1987 and may improve the model's representation of these parameters.

4.2.2. Breathing Rates

Andersen et al. (1986, 1987) determined breathing rates for humans and mice by direct observation. The value for mice is higher than EPA's standard value, while that for humans is markedly lower. The Andersen et al. value for humans is for a person at rest, but the federal regulatory agencies use a value considered typical of average human activity (almost twice as high as Andersen et al.'s value) or occupational activity (nearly three times higher than Andersen et al.'s value).

For the model used by Andersen and Reitz to apply to expected human exposures, the breathing rates would have to be increased to reflect the higher breathing rates typical of active humans. This is an important issue when comparing the federal agencies' estimates of risk based on linear extrapolation from applied dose to the results of extrapolations based on the pharmacokinetic model. In assessing the impact of the pharmacokinetic model on risk estimations, the human breathing rate factor should be kept constant and only the use of pharmacokinetic information should vary. Andersen et al. (1986, 1987) have compared the federal agencies' risk estimates (based on the higher human breathing rate and the applied dose method) to risks estimated using the pharmacokinetic model and the lower breathing rate. This issue is discussed further in the section evaluating the effect of the pharmacokinetic model on risk assessment.

4.3.3. Metabolic Parameters

The remaining parameters of the model are those that characterize how much of each metabolic pathway's activity occurs in lung versus liver tissue and how the rates of metabolism along each pathway vary with tissue concentration of DCM.

It is difficult to estimate from experiments how the relative rates of metabolism by the MFO and GST pathways vary with DCM concentration in the tissues, because the end products of DCM metabolism are not pathway-specific. In the past (Anders et al., 1978; Ahmed and Anders, 1978), DCM was thought to produce carbon monoxide via the MFO pathway and carbon dioxide via the GST pathway. If this were so, the relative production of carbon monoxide and carbon dioxide could be monitored as indicators of the relative metabolic rates of the MFO and GST pathways, respectively. Gargas et al. (1986) presented data, however, which suggest that the MFO pathway produces a substantial (but unknown) amount of carbon dioxide as well as carbon monoxide. If, in fact, carbon dioxide is produced by both pathways, its production cannot be used as an indicator of GST metabolism alone.

The question of whether or not carbon dioxide is produced by the MFO pathway has other implications as well. Crucial to the conclusion that humans, rats, and hamsters have little GST activity toward DCM is the assumption that the carbon dioxide produced by DCM metabolism in these species comes primarily (or

totally) from the MFO pathway. If the MFO pathway does not, in fact, produce carbon dioxide, the observation of carbon dioxide production in these species following DCM exposure would be inconsistent with little or no GST activity. CEFIC has underway new studies using deuterated ^{14}C-DCM, which, by exploiting the stable isotope effect, should be able to distinguish carbon dioxide produced by the MFO versus the GST pathway. These studies can be conducted in vivo at low doses and should resolve questions concerning the extent of GST metabolism at exposures well below the MFO saturation point.

Because of the difficulty in estimating metabolic rates from experimentation, Andersen et al. (1986, 1987) estimated these parameters for the model indirectly by a mathematical optimization procedure. Metabolic rates were set at the rates that optimized the model's ability to predict the loss of DCM from a closed inhalation chamber, as the compound was taken up and metabolized by mice, rats, and hamsters; i.e., values were selected that gave the best fit of the model's predictions of the rate of DCM disappearance to actual data on the disappearance of DCM from the inhalation chamber.

Although this "curve fitting" procedure may result in a good characterization of total metabolism, it is an inexact means of allocating metabolism between the MFO and GST pathways. The HRAC (1987) has shown that if the rate of metabolism along the GST pathway is reduced fourfold, for instance, from the optimum values used by Andersen and Reitz (and the MFO pathway parameters

are then readjusted), the fit of the model to the chamber data is virtually as good as before. Fairly large deviations from the "best" metabolic rate parameter values are required before this fit deteriorates appreciably. The HRAC's analysis shows that many alternative sets of metabolic parameters, implying different amounts of metabolism by the GST and MFO pathways, are supportable by the data. As a consequence, the model's crucial output with respect to risk assessment, that is, estimates of tissue level concentrations of DCM's metabolites, has an uncertainty factor of several fold.

Because it was inappropriate to obtain human data on the disappearance of DCM from an inhalation chamber, Andersen et al. estimated the metabolic rate parameters for humans by extrapolating from the values estimated for rats and mice. Andersen et al.'s extrapolation procedure may compound the uncertainty already inherent in the rodent values discussed previously (HRAC, 1987). Data from CEFIC (1986c) point to a possible large error in the model's estimate of human GST metabolism in the liver, for example.

Once the parameters describing the overall amount of MFO and GST metabolism in the body are estimated, it is necessary to specify the relative activities of the two pathways between metabolically active organs, e.g., liver and lung. Andersen and coworkers partitioned the activity of the MFO and GST pathways between liver and lung using data from Lorenz et al. (1984) on the relative activities in each tissue toward surrogate

substrates (2,4-dinitrochlorobenzene for GST activity and 7-ethoxycoumarin for MFO activity).

The surrogate substrates used may not accurately reflect the activities in each tissue toward DCM, however. On the basis of the Lorenz et al. data, Andersen et al. set the proportion of MFO metabolism occurring in the human lung at a very low level compared to the mouse lung. Lorenz and coworkers noted, however, that their human lung preparation contained endogenous inhibitors of the MFO pathway. EPA believes that a higher level of MFO activity in the lung than Andersen et al. chose is probably more realistic (HRAC, 1987). Errors in the partitioning of metabolism are considered important if only one metabolic pathway is responsible for DCM's carcinogenicity. An error in the proportion of MFO versus GST metabolic activity occurring in a particular tissue could lead to over- or underestimates of tissue-specific risks.

CEFIC (1986e) provided direct in vitro measurements of GST and MFO metabolic rate parameters in liver tissue from rats, mice, and humans. The most important of these experiments are those reporting no detectable metabolism of DCM by cytosolic preparations of hamster and human liver tissue, implying that the GST pathway operates on DCM in these tissues at a level below the limit of detection of the test system, at most. This finding is in conflict with the predictions by Andersen et al. (1986, 1987) of substantial GST metabolism in human liver.

Direct comparison of the in vitro measurements with the

Andersen et al. in vivo estimates is difficult because of the problem of extrapolating from metabolic parameters estimated on isolated cell fractions (which reflect CEFIC's work) to the rates expected in intact organs. The motivation for attempting such a comparison, despite the difficulties, is to provide a check on the metabolic parameters used by Andersen and Reitz. Preliminary work by the HRAC (1987) indicates that some metabolic parameters extrapolated from the in vitro data may disagree with the values in the model used by Andersen and Reitz by up to an order of magnitude or more, depending on the set of assumptions used in the extrapolation. Looking at GST metabolism in the human liver specifically, the GST metabolism rate may be approximately 7.5-fold below the value used in the by Andersen and Reitz, if the level of GST metabolism is assumed to be at the experimental limit of detection reported by CEFIC (1986e).

The CEFIC work and its implications for the metabolic parameters in the model used by Andersen and Reitz must be interpreted cautiously, however. The in vitro (1986e) experiment tested tissue from four human livers. CEFIC (1987c) has given the following details concerning the treatment of the human tissue samples. The samples obtained were from renal transplant donors killed in accidents. The donors, all males in the age group ranging from 13 to 34 years, were previously healthy and were not receiving any form of medication. Prior to the transplantation procedure, the donors were maintained on life support machines and given a mixture of dopamine, rogitine,

heparin, dibenzylamine, largactil, and lasix to maintain the viability of the kidneys. The livers were perfused in situ with ice-cold medium within 7-8 minutes of cardiac arrest occurring. Following removal of the kidneys, samples of liver were taken and kept on ice. Subcellular fractionation of these samples was carried out within one hour.

Viability of the livers was tested with a series of markers, namely: ECOD, EROD, ALE, LAH, and cytochrome P-450 content. Specific GST activity was not assayed at this time, but was analyzed during the DCM studies using the broad-spectrum substrate 1-chloro-2,4-dinitrobenzene. The levels measured, relative to those in animal livers, were comparable to those reported in the literature by other workers using biopsy techniques (Lorenz et al., 1984).

The DCM-specific GST in mouse and rat liver has been shown to be stable for at least one year under conditions equaling those under which the human livers were stored (-70° C). CEFIC concluded, therefore, that there is no reason to suspect that there was any loss of GST activity during the removal of the livers or the storage fractions.

It does not appear from the methodology described by CEFIC that tissue viability is an issue in considering the results of the in vitro experiments. A sample of four livers, however, provides an uncertain basis from which to generalize to all humans in view of the known polymorphism among humans in activity of GST toward certain substrates (Seidegard and Pero, 1985). The

GST enzyme is actually a complex of similar isozymes, each of which may have different activity (or no activity) toward any given substrate. The mix of isozymes varies among individuals, raising the possibility that some humans may have substantial GST activity toward DCM while others do not. At present, there is no direct evidence of variability among humans in GST activity toward DCM, however.

Also important is the insensitivity of the test system used to detect GST activity. CEFIC measured GST metabolism in vitro by monitoring the formation of formaldehyde, the final in vitro metabolite. The assay used is not strictly formaldehyde-specific and appears to have a high background level of formaldehyde formation as well, resulting in a test system capable of detecting only relatively high rates of GST metabolism. The results obtained in the CEFIC experiments should not be interpreted, therefore, as indicating total absence of GST activity. The in vitro experiments do point to lower GST activity in human liver than predicted by the model used by Andersen and Reitz, but how much lower is uncertain, at present.

CEFIC has underway new studies using an improved method of detection of the GST pathway in human and animal tissues. The new studies will determine in vitro GST metabolism using ^{36}Cl-DCM which may improve the level of detection of GST activity by up to two orders of magnitude. In a May 1987 letter to EPA, Green and Reitz stated that these studies, and similar studies conducted by the Dow Chemical Company, have in fact detected low levels of GST

activity toward DCM in human tissues. No quantitative data are yet available, however. CEFIC plans to use these data in conjunction with new in vivo experiments to arrive at estimates of human metabolic parameters that, while still somewhat indirect, are based on the only experimental data obtainable from humans. Such estimates could improve the model used by Andersen and Reitz by substituting experimentally-based values of metabolic parameters for those developed indirectly by scaling or by using surrogate substrates. CEFIC expects to make the results of their studies available in the summer of 1987.

Even if the level of GST activity is assumed to be at the level of detection of the current formaldehyde assay, however, it is difficult to know exactly how to use the in vitro data to estimate in vivo parameters, as noted above. The method of extrapolation used is to a certain degree arbitrary; it was developed in the absence of experimental data indicating the true relationship between the rates of reaction in isolated cell fractions and intact organs (HRAC, 1987). EPA considers the estimates based on this method to be fairly uncertain.

Turning next to the parameters for the human lung, CEFIC was not able to determine in vitro GST metabolism rates for this tissue, owing to a lack of availability of human tissues. The results on liver tissue in vitro are not necessarily indicative of a similar lack of activity in lung tissue. As noted previously, the GST enzyme is actually a complex of similar isozymes, each of which may have different activity (or no

activity) toward a given substrate. Although the isozymes in human liver apparently have low activity toward DCM, they are highly active when assayed with 2,4-dinitrochlorobenzene, a substrate that is metabolized by all GST isozymes. Similarly, the differences among species in the ability of liver GST to metabolize DCM are not due to differences in total GST, but to a different array of isozymes. This array of isozymic forms varies not only across species, but also among tissues; thus, the low level of GST activity (or lack of it) toward DCM in human liver does not necessarily preclude such activity in human lung tissue, or any other tissue.

Andersen et al. (1986, 1987) estimated the level of GST metabolism in human lung by assuming that the relative activity of lung and liver toward the general substrate 2,4-dinitrochlorobenzene will reflect differences in GST metabolism of DCM as well. The substrate 2,4-dinitrochlorobenzene measures the activity of all GST isozymes, and therefore this method provides a poor estimate of the ability of GST in the lung to metabolize DCM, since the lung may have a subset of isozymes different from the liver.

4.3. VALIDATION OF THE MODEL

Of more major concern than the certainty of individual parameters is whether the pharmacokinetic model as a whole can be shown empirically to be a good representation of the processes governing the body's disposition and metabolism of DCM over a range of exposures and times. For the pharmacokinetic model to

be fully validated, its output, that is, its predictions of the amount of metabolism via the MFO and GST pathways, should be measured against actual data.

Andersen et al. (1986, 1987) present several examples of their model's ability to predict concentrations of DCM in the blood at various times for mice, rats, and humans. The model's predictions fit the data fairly well, indicating that the model gives a reasonably good picture of the uptake and total body clearance of DCM. The issue of the individual rates of metabolism by the two pathways is not clarified by such comparisons, however.

Validation of the kind desired is not possible, at present, owing to a lack of data on the actual production of pathway-specific metabolites in the tissues for both pathways. Reitz et al. (1986) arrived at a rough experimental determination of the rates of the individual pathways by comparing the model's predictions of carbon monoxide and carbon dioxide production from the MFO pathway and carbon dioxide production from the GST pathway with experimentally observed levels of carbon monoxide and carbon dioxide production in mice. This comparison assumed that the ratio of carbon monoxide to carbon dioxide resulting from MFO metabolism remains fairly constant at all doses. Reitz et al. attributed all carbon dioxide production, above the level they expected from MFO metabolism, to the GST pathway. The model predicted the production of carbon monoxide and carbon dioxide within a factor of 2 for two different doses of DCM. EPA

believes, however, that the assumption concerning the proportion of carbon dioxide that comes from the MFO versus the GST pathways lacks verification.

To summarize, EPA finds that uncertainties in the model used by Andersen and Reitz arise chiefly from the input data rather than from the model's structure. Full validation of the model used by Andersen and Reitz is not possible, at present, because DCM uptake and blood level data do not distinguish between disappearance of DCM via the GST pathway and the MFO pathway. Without data on the rates of metabolism by each pathway in the tissues, a wide range of relative activities of the two metabolic pathways can be shown to fit the data on the parent compound.

In a presentation to the HRAC on February 19, 1987, Dr. T. Green outlined work underway (sponsored by CEFIC) on validating the model used by Andersen and Reitz. Dr. Green stated that the model, although apparently correct in overall structure, fails to predict much of CEFIC's data on blood levels of DCM and carboxyhemoglobin (which results from carbon monoxide production). He noted that in order for the model to more accurately predict experimental data, the fat/blood partition ratio had to be perturbed beyond the expected range of this parameter and concluded that some other parameter, in probability the metabolic rate constants, must be in error in the model. CEFIC is currently engaged in developing new experimental values for partition coefficients, tissue volumes, breathing rates, and metabolic parameters. This work is expected to be available in

the summer of 1987.

From the discussions above, it is clear that the model used by Andersen and Reitz may be improved by additional data and validation. Nevertheless, EPA believes that the structure of the model is sufficiently well developed at present to provide a means of considering the available knowledge of DCM metabolism and pharmacokinetics, as it relates to risk assessment, in a way that is not possible through the applied-dose method. While confidence in the results of the model are expected to increase upon further model validation, the development of preliminary estimates using the results of the model as currently developed provides insight into the effect on risk estimates of metabolism and pharmacokinetic information.

5. ASSUMPTIONS CONCERNING THE CARCINOGENIC PATHWAY

Fundamental to the position that knowledge gained from the pharmacokinetic model, despite its uncertainties, leads to lowered risk estimates for DCM, are the assumptions that the product(s) of only one metabolic pathway, the GST pathway, is responsible for tumorigenesis and that this pathway is less active in humans than it is in mice. CEFIC (1987a) and Andersen et al. (1986, 1987) assume this to be the case.

CEFIC conducted a series of experiments examining MFO and GST metabolic pathway activity in animals and in human tissue to determine whether species differences in metabolism could explain observed differences in carcinogenic response seen in animal bioassays. An in vivo experiment (CEFIC, 1986f) compared the exhalation rates of carbon dioxide and blood levels of DCM in rats and mice exposed to DCM at high concentrations. In this experiment, the MFO pathway was presumed to be saturated; thus, the further increases in levels of expired carbon dioxide above the levels expected from the saturated pathway were taken as an indicator of increasing GST activity at high doses. Based on the fact that rats had much higher levels of parent DCM in the blood but much lower levels of carbon dioxide exhalation than did mice, CEFIC concluded that mice have the greater capacity, by far, to metabolize DCM via the GST pathway. Results of in vitro experiments were consistent with the in vivo findings. The rate of GST metabolism by the rat liver was low, about 12 times lower

than the rate found in the mouse liver. These preliminary assays failed to find evidence of GST activity in hamster or human liver tissue. The insensitivity of the assays used prevents the conclusion that there is no GST activity toward DCM in these tissues, but the assays are sufficient to suggest that the level of activity is low.

The results of the CEFIC studies track well with the hypothesis that it is GST metabolic activity which produces the carcinogenic response observed in mice. Mice apparently use the GST pathway to a greater extent than other species tested (rats, hamsters, and humans), and, in bioassays, mice developed tumors at sites shown to metabolize DCM by the GST pathway. Rats, which developed neither liver nor lung tumors, showed only low levels of GST metabolism in the liver and no detectable rate in the lung. Hamsters, which did not develop tumors, showed no evidence of the GST pathway in either the lung or the liver. Human lung tissue has not been tested, but CEFIC reports that the preliminary studies of human liver tissue showed no measurable activity of the GST pathway toward DCM. Thus, it appears that tumor incidence correlates with GST activity.

The MFO pathway has been considered to be theoretically capable of producing reactive intermediates and, thus, potentially carcinogenic. But the available evidence does not show correlation between the rate of metabolism via the MFO pathway and the susceptibility of species to the development of cancer following exposure to DCM. The CEFIC in vivo and in vitro

experiments indicate that the MFO pathway is saturated at about the same level across the species tested; yet, in bioassays conducted at doses above the MFO saturation level, there is considerable variability in the tumor pattern across species. Moreover, in the species that did develop tumors, tumor incidence increased with increasing dose despite the fact that MFO activity was at a constant maximum level. Thus, it is very unlikely that the MFO pathway is the source of these tumors. Although, conceivably, a very low level of carcinogenic activity on the part of the MFO pathway is a possibility, there is virtually no experimental evidence to support it.

The Andersen et al. (1986, 1987) comparison of the results from the National Coffee Association (NCA) study (1983) and the NTP (1985) mouse bioassay also implicates the GST pathway. Andersen et al. (1986, 1987) determined that mice in the NCA bioassay, which were exposed to relatively low levels of DCM, had levels of MFO metabolic activity (both in the liver and lung) similar to the levels found in mice exposed to high doses of DCM in the NTP bioassay. The fact that tumors were observed in the NTP mice but not in the NCA mice (at a statistically significant increase), indicates that the MFO metabolites could not have been responsible for the excess tumors in the NTP mice. Andersen et al. concluded that these must then have been produced either by the parent compound or by the GST metabolites.

The parent compound was not considered a likely candidate for several reasons. DCM is relatively inert and does not have

direct alkylating activity. Its mutagenicity in the Ames test appears to be due to bacterial metabolism, and is enhanced after activation by cytosolic GST (Green, 1983). Moreover, the appearance of liver tumors in animal studies does not correlate well with parent DCM concentration; in the NTP bioassay, the rat liver showed no tumor response despite the presence of DCM in concentrations higher than those in mice (in which a liver tumor response was observed).

CEFIC (1986a) also looked for evidence from the mouse lung where cell toxicity appears to be most intense in certain specialized cells known as Clara cells. Clara cells are thought to have a high potential for metabolizing DCM by the GST pathway. If, in fact, DCM itself were the toxic species (with metabolism acting to detoxify it), one would expect to find a significant response in all of the lung's cells rather than in the Clara cell, where metabolism appears to occur more quickly.

Andersen et al. (1986, 1987) and CEFIC (1987a) are reasonably certain, based on the evidence, that the GST pathway is the sole route producing a carcinogenic response. A few points of caution should be noted, however. The mechanism by which DCM causes carcinogenesis may not involve DNA alkylation; the lack of alkylating activity does not, by itself, eliminate parent DCM as a suspect. The Clara cell response could be due to some membrane phenomenon allowing easier penetration of DCM itself into this cell type. Further, the argument concerning the Clara cell assumes that the cytotoxic response given by the Clara

cells is a precursor to tumor formation, which has not been established. Exposures to other chlorinated solvents (tetrachloroethylene for example) lead to similar Clara cell lesions without subsequent tumorigenesis.

But although it is possible to argue with some of the individual lines of evidence, collectively the evidence makes a strong case implicating the GST pathway. EPA concludes from the weight of evidence that DCM's carcinogenicity is most likely produced as a result of metabolism via the GST pathway. Parent DCM and the MFO pathway are considered to be possible rather than probable sources of tumorigenic potential. Neither one correlates with tumor incidence, either across species or across doses. Further, the parent compound is thought to be chemically unreactive.

6. ASSUMPTIONS CONCERNING THE MECHANISM OF ACTION

Before proceeding to a discussion of the implications of the pharmacokinetic model and theories concerning the carcinogenic pathway for quantitative risk estimation, the relevancy of the response seen in mice to humans must be addressed. It has been proposed (CEFIC, 1986a, g) that the response of mice to DCM may be limited to that species based on hypotheses about the mechanism by which DCM causes cancer. If DCM can be shown to cause cancer in laboratory animals by a mechanism that is lacking in humans, there is no evidence of a cancer risk to humans. The genotoxicity and cytotoxicity of DCM have both been investigated as possible mechanisms of carcinogenic action.

6.1. GENOTOXICITY

6.1.1. Bacteria, Yeast, and Drosophila

Reviews of the evidence as to the genotoxicity of DCM (U.S. EPA, 1985a; United Kingdom Health and Safety Executive, 1985; CEFIC, 1987a) lead to the conclusion that DCM is mutagenic, with and without metabolic activation, in most bacterial assays for point mutation (Salmonella typhimurium, Escherichia coli). The United Kingdom Health and Safety Executive (UKHSE, 1985) noted that, as S. typhimurium is capable of converting DCM to a mutagenic chemical species, the positive results obtained without an exogenous metabolizing system do not indicate that DCM is a direct-acting mutagen. Rather, while DCM does not appear to require activation by mammalian liver microsomes, the chemical

must still be metabolically transformed in order to obtain mutagenic activity. Results of point mutation and mitotic recombination tests in yeast (Saccharomyces cerevisiae) are mixed; negative and positive responses were given up to toxic doses (360,000 ppm), at which one strain showed a clear positive response. Sex-linked recessive lethal mutation assays in Drosophila also gave mixed results; only one out of four studies of recessive lethal mutations in Drosophila reported a positive result (Gocke et al., 1981) and the reliability of this study has been questioned (CEFIC, 1986b) because of problems in the methodology and the weakness of the response observed.

6.1.2. Mammalian Cells in vitro

Studies on chromosomal damage have shown DCM to be clastogenic, with and without the addition of a metabolic system, in mammalian cells in culture. When tested for its ability to induce transformation in a variety of cell systems, DCM gave negative results in tests of mouse cells and in most tests of rat cells (U.S. EPA, 1985a; UKHSE, 1985; CEFIC, 1987a). It tested positive in one assay of Syrian hamster cells (Hatch et al., 1983). In vitro studies of unscheduled DNA synthesis (UDS), indicative of DNA repair, provided negative results in most assays of rat hepatocytes, human fibroblasts, and human lymphocytes (U.S. EPA, 1985a; UKHSE, 1985; CEFIC, 1986b). CEFIC reports (1987a) that a "marginal" positive result was observed in a rat primary hepatocyte UDS study (Thilagar et al., 1984), but notes that details of the study are not available. Chromosomal

mutation assays gave mixed results; only one study (Thilagar and Kumaroo, 1983) of Chinese hamster ovary cells can be judged positive.

6.1.3. Mammalian Cells in vivo

In vivo mutagenicity assays testing DCM in both rats and mice have given uniformly negative results, including a recently completed mouse micronucleus test at gavage doses up to 4000 mg/kg (CEFIC, 1986b). Results in other test systems have been also, for the most part, negative. Recent work from CEFIC (1986c) on UDS in rat hepatocytes from rats exposed in vivo gave negative results, as did studies of DNA binding of DCM in rat and mouse liver and lung (CEFIC, 1986d). CEFIC's UDS experiments with B6C3F1 mice gave limited but statistically significant evidence of the induction of mitosis in the liver. CEFIC notes (1987a) that further experiments are in progress to establish the biological significance of this effect.

The CEFIC in vivo studies have been criticized (HRAC, 1987) on the grounds that, given the short exposure periods, doses tested (2000 or 4000 ppm of DCM by inhalation for periods up to 6 hours) were below the level which might have given a positive response. EPA does not, therefore, consider the results of these in vivo studies to be definitive.

6.1.4. Summary

Reviews of the full range of genotoxicity studies of DCM (U.S. EPA, 1985a; UKHSE, 1985; CEFIC, 1987a) have concluded that there is clear evidence of mutagenicity in bacteria, but no clear

and consistent evidence of mutagenicity in mammalian systems. DCM has been shown to be mutagenic in bacteria, gave mixed results in tests of yeast, Drosophila, and mammalian cells in culture, and gave largely negative results in mammalian cells in vivo. CEFIC (1987a) hypothesizes that the positive mutagenic response of bacteria are caused by the metabolism of DCM to short-lived proximate mutagens, e.g., formyl chloride and S-chloromethyl glutathione. They consider these species to be so chemically unstable as to be unlikely to survive long enough to affect DNA protected by a nuclear membrane in the nuclei of higher organisms. Scientific evidence to support this hypothesis is lacking, however.

Given the evidence of in vitro clastogenicity and the insensitivity of the in vivo UDS and DNA binding studies, EPA concludes that DCM may be a weak mutagen in mammalian systems, weak enough to be below the level of detection of the in vivo studies. A weak genotoxic mechanism is considered to be a possibility due in part to the failure, at present, to identify an alternative mechanism of action for DCM, as discussed in the following section.

6.2 ALTERNATIVE MECHANISMS OF CARCINOGENIC ACTION

CEFIC (1986a, b, c, d) has addressed the question of whether DCM acts through an epigenetic mechanism (e.g., cell toxicity leading to tumor promotion) rather than through genotoxicity. A 10-day inhalation study to investigate the effects of DCM on the rat and mouse liver and lung showed no significant toxic effects,

in mouse or rat livers or in the rat lung. Some transient cytotoxic effects, including cytoplasmic vacuolation, were observed in the Clara cell of the mouse lung, apparently brought on by MFO metabolites, but the significance of this response to potential carcinogenicity is unclear (CEFIC, 1987a; HRAC, 1987). Similar responses to other solvents (e.g., tetrachloroethylene) are not associated with tumor production. CEFIC has hypothesized that the destruction of smooth endoplasmic reticulum in the Clara cells results in reduction of MFO activity, which in turn leads to greater GST metabolism than would otherwise be expected. If the GST products are not genotoxic, it is unclear how such metabolism would enhance tumorigenicity since cytotoxicity alone appears to be insufficient.

It is also unclear whether carcinogenesis induced through cytotoxicity would be specific to the mouse lung. The histogenesis of Clara cells, which are more prevalent in mice than other species, and type II pneumocytes, which may be more prevalent in other species (including humans) than in mice, are quite similar. Clara cells and type II pneumocytes are both active secretory cells and may be similar biochemically. Thus, any process that produces a response in the Clara cells in mice must be suspected of producing a similar response in the type II pneumocytes in other species as well.

Seeking information on the mechanism of action in the liver, CEFIC (1986g) examined the possibility that DCM induces a carcinogenic response in the mouse liver through increased cell

turnover. In an S-phase hepatocyte study, small, variable increases in the incidence of S-phase cells after DCM exposure were observed. CEFIC concluded that although these increases were statistically significant, the biological significance of such small changes is unclear.

It is widely agreed (CEFIC, 1987a; HRAC, 1987) that much uncertainty remains about the mechanism of action of DCM. There is little evidence at this point to support either a genotoxic mechanism or some epigenetic effect such as cytotoxicity, or to indicate that the mechanism is limited to mice. Additional data on mechanism may be furnished by the National Institute of Environmental Health Sciences (NIEHS) which plans to investigate further the role of cell replication in DCM tumorigenesis and the pattern of oncogene activation in spontaneous and dose-related tumors, and by CEFIC which is conducting studies to evaluate the effects of DCM in the Clara cell in relation to tumor development.

7. IMPACT OF THE PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL USED BY ANDERSON AND REITZ ON HUMAN RISK ESTIMATES

In comparing risk estimates based on the physiologically based pharmacokinetic model to EPA's risk estimates derived from the applied-dose procedure, Andersen et al. (1986, 1987) concluded that EPA's method overestimates internal dose (and consequently risk) by a factor of 167 for the human liver and 144 for the human lung. (The internal dose is taken in this comparison to be the amount of metabolite production per liter of tissue by the GST pathway.) These factors actually represent the combined effect of several components, only some of which EPA feels are appropriate to the comparison. The following section separates the components and examines them individually.

The factors, as Andersen et al. derive them, comprise the following: (1) factors of 13.5 and 11.3 for liver and lung, respectively, representing the non-proportionality between applied dose (mg/kg/day) and internal dose (mg-equivalents of GST pathway metabolism/L/day) in these tissues; and (2) an additional factor of 12.7, which Andersen et al. attribute to an arbitrary "interspecies correction factor" applied by EPA to account for anticipated species differences in pharmacokinetics for DCM (13.5 x 12.7 = 167; 11.3 x 12.7 = 144). EPA finds the differences between risk estimates derived by the two procedures to be far less. The disagreement stems from two factors: (a) breathing rates, and (b) the appropriate application of the "surface area

correction" to dose.

In EPA's Addendum to the Health Assessment Document for Dichloromethane (1985b), applied doses are calculated as the amount of DCM breathed in per kg of body weight per day, estimated using empirically-based breathing rates (m^3/day) and assuming 100% absorption. Because larger animals breathe less air per unit of body weight, humans receive a smaller applied dose from a given exposure to a certain vapor concentration than do mice. The model used by Anderson and Reitz incorporates the fact that humans have a smaller input of DCM per kg than mice, but the model uses a set of breathing rates different from those used by EPA. The model's value for human breathing rate (12.5 m^3/day) was measured for a man at rest, and is consequently much lower than EPA's estimate (20 m^3/day) which is based on average daily activity. When the model is used in assessing risks from actual human exposures, its parameters should reflect normal human activity levels.

Further, the model's breathing rate value for mice (0.084 m^3/day) is much higher than EPA's estimate (0.043 m^3/day). Andersen et al. (1986, 1987) compared the results of their model to EPA's procedure without accounting for the fact that the two methods use different breathing rates, both for the mice and for humans. A more accurate comparison is achieved when both methods use the same breathing rates. If the Andersen et al. values (12.5 m^3/day for humans and 0.084 m^3/day for mice) are used in both EPA's applied dose and the calculations used by Anderson and

Reitz, the non-proportionality between applied dose and internal dose is only a factor of 4.3 in liver and 3.6 in lung, rather than the factors of 13.5 and 11.3, respectively, implied by Andersen et al.'s comparison. (The decrease of 3.1-fold from the factors in the Andersen et al. comparison reflects the difference in the ratio of mouse to human breathing rates between the model and EPA's assumptions. If the EPA breathing rates are adopted in both the model and EPA's calculations, the non-proportionality of applied and internal dose is somewhat larger, 7.7-fold for liver and 9.4-fold for lung. Substitution of the EPA breathing rates for the Andersen and Reitz breathing rates does not lead to a proportional change in internal dose estimates, hence the difference in the two sets of estimates. For the sake of clarity in analyzing the Andersen et al. comparison of the EPA applied-dose risk estimates and the pharmacokinetic model-based internal dose risk estimates, further discussion of the comparison incorporates the Andersen and Reitz set of breathing rates. But when the pharmacokinetic model is used to actually generate risk estimates, EPA uses the EPA set of breathing rates. These calculations are discussed further in Chapter 8.)

Andersen and coworkers note that EPA assumed the human dose (in mg/kg/day) producing a given risk level to be lower than the mouse dose producing that same risk by a factor of 12.7. This assumption is known as the "surface area correction," and corresponds to the assumption that it is the amount of applied dose per unit of surface area, rather than per unit of body

weight, that is equivalent in risk across species. Andersen et al. interpret this to mean that EPA expects internal doses to be 12.7-fold higher in humans for a given applied dose, with risk being directly proportional to tissue concentration, independent of species. The higher internal dose in humans is presumably expected owing to pharmacokinetic differences between species that arise as a result of the scaling of key pharmacokinetic parameters in proportion to body surface area rather than to body weight.

In other words, Andersen et al. have interpreted the surface area correction as a correction to applied dose to produce an estimate of anticipated internal dose. They show that, when internal doses are actually estimated with their model, they are not in fact 12.7-fold higher in humans, as anticipated, but rather an estimated 4.3-fold* lower in liver and 3.6-fold* lower in lung. To Andersen et al. (1986, 1987) this implies that use of the surface area correction is incorrect for DCM, and that EPA's risk estimates are too high by 12.7 x 4.3 = 54-fold* and 12.7 x 3.6 = 46-fold* for liver and lung, respectively. (Their paper cites the figures as 167-fold and 144-fold since the 3.1-fold inflation due to breathing rates differences had not been corrected for).

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*These numbers represent estimates based on the model and assumption for breathing routes used by Andersen and Reitz. When EPA's breathing rate assumptions are used in the pharmacokinetic model, these factors become 7.7 for liver and 9.4 for lung without the surface area correction factor; 97.8 (7.7 x 12.7) for liver and 119.4 (9.4 x 12.7) for lung with the surface area correction factor.

In summary, EPA finds that Andersen et al.'s estimate of 167-fold difference between liver cancer risks (for example) estimated from the applied-dose method and the pharmacokinetic model can be attributed to the following factors:

Pharmacokinetic nonlinearity (as predicted using the model)	4.3-fold
Use of different breathing parameters (by Andersen et al. versus EPA)	3.1-fold
Surface area correction	12.7-fold
Composite	167-fold

It should be clear that only the factor of 4.3 comes from the model used by Andersen and Reitz. That is, when comparing mice at high bioassay doses to humans at low exposure levels, the non-proportionality of applied dose to internal dose amounts to only a few fold. Although the model reveals some differences between species and from high to low doses in the proportion of an applied dose of DCM that is metabolized by the GST pathway, these differences are apparently not large.

The other two factors are features of the applied-dose procedure, against which the model's results are being compared. That is, they are part of the question about how much impact the finding of a 4.3-fold non-proportionality between applied and internal dose has on risk estimation. The validity of their inclusion in the calculation of the impact of the model used by

Andersen and Reitz on risk extrapolation rests on whether they in fact correctly represent the use of applied doses by EPA. As noted previously, EPA believes that the 3.1-fold factor that arises from the use of different breathing rates in the applied dose calculations and in the model is an extraneous factor, and should be eliminated from the comparison by using a single set of breathing rates.

This leaves for consideration the contentious interspecies correction factor which, in the EPA procedure, is equal to the relative surface-to-volume ratios of mice and humans, giving a factor of 12.7 by which human applied doses in mg/kg/day are divided to be of equal lifetime risk to mouse doses. The question revolves around what the use of this factor implies about the expectations for the underlying pharmacokinetics, the relation of applied to internal dose, and the reasons for interspecies differences in carcinogenic potency. EPA feels that the surface area correction is not simply a correction on applied dose to account for an expected higher internal dose in humans, as presumed by the analysis of Andersen et al. (1986, 1987). That treatment oversimplifies a complex issue, which is discussed in the following chapter.

8. USING INTERNAL DOSES AS A BASIS FOR HUMAN RISK ESTIMATION

The interspecies correction factor is applied to account for the difference in expected potency of a given applied dose in experimental animals and humans. Many biological differences exist among species that can be expected to enter in to such differences in potency. Compared to mice, humans have a slower excretion rate of compounds from the body, their rates of metabolism are generally slower, and they have a lower rate of cell division. They may have more efficient DNA repair and may be more effective in scavenging free radicals and other reactive compounds. On the other hand, they have many more cells at risk, only one of which need undergo carcinogenic transformation to produce a tumor, and they may be exposed to carcinogens for a much longer lifetime. Many other potential factors could be named. Some, but not all, of the above factors are pharmacokinetic; that is, they can be expected to influence the relationship of internal doses in the tissues to the amount of dose applied. Other factors, however, can be expected to be manifested in species differences in the degree of carcinogenic responsiveness of the tissues to a given internal dose. The potential contribution of these other factors continues to be problematic even after interspecies pharmacokinetic differences are accounted for. It is clear that, even when internal dose information is available, no matter how reliably known, there remain important questions about the degree to which different

species will respond to those internal doses.

The interspecies extrapolation factor must take into account the combined effect on carcinogenic potency of all underlying biological differences between species. The few data that exist (Crump et al., 1985; Allen et al., 1986) suggest that fairly good estimates of human risk are achieved (on average) when applied doses are scaled according to some measure of body size (surface area or weight) and/or some measure of life span. No one factor in the mechanism of carcinogenesis can be identified as the key to interspecies differences in potency, however. Since pharmacokinetic data address only part of the interspecies extrapolation question, such data alone cannot solve the difficulties of extrapolating risks across species. Some elements of the former assumptions must be retained in the extrapolation procedure, even when internal dose estimates are available. The question is, how much of species differences in potency (if any) can be ascribed to species differences in pharmacokinetics, and how much (if any) can be ascribed to the remaining factors that lead to differences in "sensitivity" of the tissues?

The disagreement about how and when to apply the surface area correction factors to applied dose illustrates that there is some question about the basis for specifying those pharmacokinetic differences between species implicit in the surface area correction procedure (i.e., the differences expected as a consequence of the differences in scale rather than to

chemical-specific metabolic factors, saturation of metabolism, and so on). Using internal dose estimates in place of applied doses in risk extrapolation should change the risk estimates by the degree that the original assumptions about pharmacokinetics, implicit in the applied-dose procedure, are shown by the new data to be in error. The impact of pharmacokinetic data, then, depends on one's view of what can be said about the prior presumptions about pharmacokinetic differences between species, and how those presumptions relate to the use of the surface area correction on applied dose. To discuss this, the rationale for the use of surface area scaling must be examined.

One way to develop an interspecies extrapolation factor is to examine the problem conceptually, from first principles. Although rodents and humans share a basically similar mammalian anatomy, physiology, and biochemistry, they differ considerably in size. The field of allometry studies the way in which the magnitude of different features varies with animal size (e.g., Schmidt-Nielsen, 1984; Mordenti, 1986). While many features tend to scale across species in proportion to body volume (or weight), others, including many rates of physiological processes, tend to scale in proportion to an animal's body surface area. The aim of the allometric approach to interspecies extrapolation is to discover what pharmacokinetic differences are expected between rodents and humans solely as a result of their differences in size.

A second way to approach the interspecies extrapolation

factor is as an empirical correction. That is, justification for the use of a particular factor (such as the surface area correction) comes through the extent to which it can be shown to be successful in predicting human cancer potencies from those determined in animal experiments. Such comparisons require epidemiologically based direct estimates of human risks against which to test the predictions from animals. It is not necessary to specify or understand the underlying biological processes that produce interspecies differences in cancer potency--only the overall combined effect, which comprises unknown contributions of pharmacokinetics and species differences in responsiveness, is assumed to be given by the surface area correction.

These two approaches lead to somewhat different methods for incorporating pharmacokinetic data into risk extrapolation, which are outlined below.

8.1. METHOD 1: COMPARISON OF INTERNAL DOSES TO ALLOMETRIC EXPECTATION

Surface area scaling of doses is routinely used in the area of predicting appropriate human dosage levels of experimental drugs from data on their effect and toxicity in experimental animals. The allometric rationale, which is supported by experimental evidence (e.g., Dedrick et al., 1970; Dedrick, 1973), is that the volume of distribution of a drug tends to scale between species in proportion to body weight, while the rate at which the drug can be cleared from the body (i.e., removed by excretion and/or metabolism) tends to scale up in

proportion to body surface area. Larger animals (such as humans) have a smaller surface area to volume ratio than smaller ones. Compared to a 35-g mouse, for instance, a human must clear drug from a volume some 2000 times larger, but the rate of clearance (in mL of blood per minute from which drug is removed) is only 159 times faster. The result is that a given initial blood concentration attenuates much more slowly in the human, and the area under the blood concentration-time curve (which is the integrated or cumulative amount of exposure of the tissues) is larger. Scaling the applied dose by surface area results in a smaller initial blood concentration in the human, but a similar total tissue exposure (area under the curve) as is experienced by the mouse. It is this rationale for surface area scaling of applied dose that is attributed to EPA by Andersen et al. (1986, 1987).

The above argument concerns exposure to the parent compound, but carcinogens are often thought to require metabolic activation to a more reactive chemical form; it is exposure of the tissues to such metabolically activated species that is at issue. A somewhat different rationale for surface area scaling, also arising from allometric observations, is based on the idea that metabolic rates can be expected to scale across species in proportion to surface area (Davidson et al., 1986). It has been argued (e.g., Ramsey and Gehring, 1980) that humans are thereby expected to have a lower rate of activation of procarcinogens, and hence should be less--rather than more--responsive to a given

concentration of parent compound, as the usual use of surface area scaling of applied doses suggests.

It should be pointed out, however, that both the slower clearance of parent compound by humans, as well as its slower metabolism to activated carcinogen, are occurring simultaneously. Humans may have a lower metabolic rate constant than mice, but the rate of metabolism depends both on this constant and on the concentration of parent compound. In fact, if it is assumed that both metabolism and non-metabolic clearance scale as surface area (as is presumed by their use in providing a basis for the surface area correction), their individual "surface area corrections" cancel out. That is, humans have a greater area under the blood concentration-time curve for a given applied dose, but this exposure to parent compound is not the relevant one. The rate of carcinogenic activation of this parent compound by metabolism is lower (per kg) than in mice for a given parent compound concentration in the tissues, but that concentration stays high longer, owing to the slow clearance, with the result that the same fraction of a dose is metabolized in each species.

Another way to view the same phenomenon is to realize that metabolic and non-metabolic clearance (i.e., excretion) are both acting to remove parent compound from the body; in fact, all of the compound introduced into the body must eventually find its way out through one or the other process. They both act more slowly in humans, since they are both allometrically related to surface area. But, so long as they both scale the same way, the

ratio of metabolic to non-metabolic clearance is the same in mice as in humans. The same proportion of an administered dose will end up being metabolized in each species, although the process will take longer to complete in humans.

The case of inhalation exposure is slightly more complex, but the same principle applies. In inhalation exposures of sufficient duration, a steady-state blood concentration is reached in which input of new parent compound is offset by loss through metabolism and excretion (which, as is the case with DCM, is usually through the lungs). Cardiac output, minute volume of breathing, and rate of metabolism (for a given parent compound concentration) all tend to scale allometrically in proportion to a species' surface area. (Actually, the argument requires only that all the factors scale in the same way, be it surface area or otherwise, but scaling close to surface area is empirically observed.) Under these circumstances it can be shown that, while breathing a given vapor concentration, steady-state blood concentrations of parent compound are expected to be equal in all species. The amount of parent compound metabolized into the active carcinogen per kg of body weight per unit time is less in humans (by the amount of the surface area correction), owing to their smaller metabolic rate constant. However, the applied dose experienced by a human breathing a given air concentration for a given time is also smaller than that of a mouse, and by the same amount, owing to the lower breathing rate per kg in humans. (As noted earlier, applied dose is figured by multiplying breathing

rate by air concentration and duration of exposure, giving the amount, in mg/kg, that is inhaled during the course of exposure.)

In other words, under a variety of circumstances, from bolus dosing to continuous inhalation, the balance of metabolic and non-metabolic clearance leads to an equal proportion of an applied dose being metabolized in rodents and humans. Focusing on the scaling of metabolic rate constants while ignoring the parent compound concentration (or vice versa) looks at only one element of the two interacting processes.

Of course, it is not necessarily true that the important rates all scale across species in just the same way, i.e., in proportion to surface area. For example, mice breathe somewhat more air per minute than predicted by strict surface area scaling, and many physiological measures scale closer to the 0.75 power or the 0.59 power of body weight than to the 0.67 power. Furthermore, peculiarities in species with regard to the properties of their metabolic enzymes, saturation of metabolism at higher doses, shifts among alternative metabolic pathways, and the like, will all cause some deviations from the pattern outlined above. Rate constants of specific metabolic pathways may not scale in the same way as the overall metabolic process. But the question here is about the a priori expectation for the relation of applied to internal dose across species. The relevance of surface area to the question of choosing an interspecies scaling factor to be used on applied doses comes from the idea that crucial elements of the physiological

processing of an applied dose may have this allometric relationship to body size. The above argument suggests that, considering the way this scaling is usually conceived, applied dose and internal dose ought to be directly proportional across species. In other words, differences in body size do not, in themselves, result in any inherent difference among species in the proportion of an applied dose that is metabolized, all else being equal. The extent to which actual pharmacokinetic and physiological data show all else not to be equal is the extent to which the risks estimated under the applied dose basis for extrapolation ought to be changed.

It is interesting to note that the model used by Andersen and Reitz predicts internal doses that very closely adhere to the argument developed above. According to the model, humans at 4000 ppm have a ratio of applied to internal dose (using the model's breathing rates) that is 0.60 times that of mice in liver tissue, and 1.6 times that of mice in lung tissue. That is, the a priori assumption that applied and delivered dose are in the same ratio across species appears to differ by less than a factor of 2. At low doses the model's predicted difference reaches about a factor of 5 in the liver, but the ratio for the lung is virtually equal to the assumption of 1.0. Thus, despite the lack of adherence to strict surface area scaling assumptions, saturation of metabolism, multiple metabolic pathways, and so on, the a priori expectation appears to be incorrect by only a small amount.

In view of the expectation of internal doses in mice and

humans outlined above, how is the surface area correction on applied dose to be regarded? It is not a correction to account for presumed differences between species in internal dose, since internal dose is expected to be a constant proportion of applied dose. Rather, it can be viewed as a correction to the expected species differences in risk from a given internal dose. That is, the assumption that humans have an equal cancer risk from a dose that is 12.7-fold smaller per kg of body weight can be ascribed, to a greater "responsiveness" to that internal dose. (Actually, one must keep in mind that risks are presumed equal for lifetime exposures at a daily rate that is 12.7-fold lower in humans. Since humans live some 35 times longer than mice, their total cumulative exposure leading to the same presumed risk is actually 35/12.7 = 2.8 times greater than for mice. Thus, the assumption is that they are slightly less sensitive than mice to a given lifetime tissue-level exposure.)

Why should one assume a 12.7-fold greater "responsiveness" to internal dose in humans than in mice? The assumption that applied doses ought to be scaled by surface area to be of equivalent risk has been used by EPA for over a decade on many chemicals, including most of those to which DCM is being compared when possible substitution of a chemical with less risk is considered. If the above argument that the general scaling principles that have been invoked in fact lead to an a priori expectation of internal doses being proportional to applied doses, even across species, then the last decade's use of the

surface area correction to predict expected human risks has been, in practice, a correction for the relative tumorigenicity across species of a given internal dose. This is true even if the stated reason for the surface area correction has been that it corrects for "metabolic differences" and some other factor. Since pharmacokinetic data on DCM provide no new information on species differences in responsiveness, the original de facto assumption ought to remain unchanged. In other words, the factor of 12.7 should continue to be applied to internal doses from the pharmacokinetic model because this corresponds to the assumption that has in effect been used all along. There is no information to justify changing this assumption at this time.

One may, of course, question whether the assumption is correct. There are some reasons to think that it may be reasonable. As noted above, it nearly corresponds to the assumption that humans and mice are equally sensitive to a total cumulative lifetime dose in mg/kg (it is off by a factor of only 2.8 from this). One may also note that some of the components of tissue sensitivity to a carcinogen, such as cell division and turnover rates, DNA repair rates, scavenging of free radicals, and so on are related to tissue aging rates and to life span. Boxenbaum (1983, 1984) relates life span and aging to a different scale of "physiological time" for each species, which tends to vary across species in approximate proportion to body surface area. These observations are not the reason for adopting a surface area factor for species differences in responsiveness--

that reason is the historical precedence cited above. But these observations do indicate the sorts of factors that might be examined to reach further understanding of tissue sensitivity. If new data become available to alter this assumption, then risk calculations using internal doses may be changed accordingly, but so must risks calculated using the applied-dose procedure.

Returning to the particular case at hand, how should the results of the model used by Andersen and Reitz alter EPA's estimation of human risk from the levels calculated using applied doses? Under the allometric view of interspecies extrapolation, and assuming that metabolism by the GST pathway at the target tissue is the relevant internal dose measure, one should simply replace the applied dose with the internal dose in the usual extrapolation procedure. To the extent that the model predicts internal doses that are not in fact proportional to applied dose, the resulting risk estimates will change. That is, both species differences in the proportion of the applied dose that is metabolized and high- to low-dose differences in this proportion affect the low-dose human risk estimate. The steps are: (a) use the model to estimate internal doses received by mice in the NTP bioassay; (b) fit a dose-response curve by the usual methods, using the NTP mouse responses and these internal doses; (c) use the model to estimate internal doses in a human subjected to a low-level continuous exposure, say 1 ppm; (d) scale that internal dose by the surface area correction; and (e) calculate the risk from that scaled dose, according to the dose-response curve.

The scaling in step (d) is applied not to alter the estimate of human internal dose, but to account for the presumed greater risk that the dose engenders in a human vis-a-vis a mouse. That is, the correction is applied in the same way as for the applied dose procedure, as a correction for species differences in responsiveness to a given dose (internal or applied).

The actual calculations of this method are shown in the HRAC (1987, Chapter 7) report. The model used by Andersen and Reitz, as presented by Andersen et al. (1986, 1987), was followed with one exception: EPA believes that its long-standing assumptions about breathing rates better reflect the general activity levels of both mice and humans than do the rates used in the model as presented. The mouse and human breathing rates in the model were adjusted accordingly. Cardiac output was then adjusted in the same proportion as the breathing rates.

The lung and liver do not generate equal amounts of metabolites during an exposure, so it is necessary to extrapolate risks for each organ separately. The risk based on mouse lung tumors is slightly over twice that based on liver tumors, despite the fact that the liver has much higher internal doses, owing to its higher metabolic activity. In fact, the risk in lung tissue per unit of internal dose is about 20-fold higher than for liver tissue. In mice used in the NTP bioassay, the occurrence of liver and lung tumors was independent; developing a tumor at one site did not affect the statistical probability of developing a tumor in the other tissue. If this is also true in humans, then

the overall unit risk can be derived by simply adding the tissue-specific unit risks. This process yields a human incremental risk for continuous inhalation of 1 ug/m^3 of 4.7 x 10^{-7}. This unit risk is 8.8-fold lower than EPA's published unit risk based on applied dose (4.1 x 10^{-6} per ug/m^3), which is based on the same NTP female mouse bioassay data.

The above analysis extrapolates both across species and across doses using the internal dose estimates from the model used by Andersen and Reitz. The difference in estimated carcinogenic potency of DCM between the NTP bioassay doses to mice and the lower doses to which humans are exposed is influenced by both species-to-species differences in metabolism (which are minor in the model as currently constituted--the results nearly correspond to the a priori assumption based on allometry) and high- to low-dose differences in metabolism within humans, resulting from the saturation of the competing MFO pathway.

One of the more uncertain parameters in the model used by Andersen and Reitz is the first-order rate constant k_F, describing GST metabolism. The human value of k_F cannot be estimated directly, and so Andersen et al. (1986, 1987) derived an estimate based on allometric scaling from observed values in rodents. (These authors used body weight to the 0.7 power, rather than the 0.67 power corresponding to a strict geometric surrogate measure of surface area for similarly shaped objects.) Thus, this element of the human model is essentially filled with

the a priori assumption of the allometric approach, which results in the model's prediction that approximately equal proportions of an applied dose are metabolized by the GST pathway in mice and humans. (Most of the 8.8-fold lowering of estimated risk that emerges from method 1 is attributable to high- to low-dose non-linearities in metabolism by the human GST pathway, rather than to species differences in metabolism). The CEFIC data on in vitro GST metabolism in human liver fractions suggest that in the model used by Andersen and Reitz, the scaled value of k_F for humans may be a good deal too high, however. If this proves to be the case as more reliable data become available, it would serve as an example of chemical- and species-specific deviations from that pattern of metabolic variation that is expected as a result of allometry. The human risks calculated by extrapolating across species on internal dose would change in approximate proportion to the change in the model's value of k_F. That is, human risks would be lowered to the degree that humans metabolize a smaller proportion of an applied dose by the GST pathway than do mice.

As discussed previously, gauging the quantitative impact of the currently available CEFIC data is difficult, since they are in vitro data that do not directly yield an estimate of the in vivo value of k_F. Furthermore, there is some question about the limit of detection of the assay. These issues prevent the use of the current CEFIC data as a basis for estimating the human k_F directly, rather than by the scaling procedure used by Andersen

et al. (1986, 1987). Nonetheless, a preliminary estimate suggests that a lowering of the human k_F by at least sevenfold or so may be indicated, which, if correct, would lower human risk estimates by about the same degree. If human GST metabolism is in fact well below the limit of detection of these studies, rather than at the limit, then further proportional lowering of human risk may be indicated. New studies from CEFIC, expected this summer, will examine human GST metabolic activity toward DCM with a much more sensitive in vitro assay in both liver tissue and (for the first time) in lung tissue. CEFIC intends to use these studies in conjunction with new in vivo work to develop more direct estimates of human GST metabolism in vivo, which can then be used (along with other new parameter estimates) to more reliably formulate the model's representation of interspecies differences in metabolism of DCM. Extrapolation of risk to humans, when calculated by the extrapolation method described above, will vary from current estimates in approximate proportion to the degree that mice and humans are shown to metabolize a different proportion of a delivered dose.

8.2. METHOD 2: USE OF PHARMACOKINETICS ONLY FOR HIGH- TO LOW-DOSE EXTRAPOLATION

Interspecies extrapolation is considered to be a problematic conversion. It is difficult to specify how a given metabolic difference should be combined with a host of non-pharmacokinetic factors in determining carcinogenic potency differences between mice and humans. If uncertainty exists about the assumptions as

to how this should be done, one can turn to a second method of incorporating pharmacokinetic considerations into risk assessment. This method (Method 2) presumes that the current lack of understanding of interspecies differences in tissue sensitivity precludes using internal doses to extrapolate across species, but assumes that the results of the pharmacokinetic model can be used for high- to low-dose extrapolation.

All extrapolations between species must make some assumptions regarding the dosimetry between species. Some factor (F) is taken as a measure of interspecies correspondence. In the case of extrapolation performed on an applied dose basis, F is based on the fact that differences between species result from differences in both pharmacokinetics (PK) and tissue response or pharmacodynamics (PD). The EPA and CPSC have traditionally based the value of F on the differences between the surface areas of different animal species (HRAC, 1987, Chapter 7). The FDA, on the other hand, bases the value of F on the differences between the weights of different animal species. Other values of F could be selected, for example, the differences in organ weight or total protein mass of a tissue. Regardless of the particular basis for F, once pharmacokinetic differences are accounted for by models, some adjustment of F may still be needed. Obviously, if F is due to differences in PK and PD and if either PK, PD, or both are adjusted by accurate quantitative means, then F must also be adjusted. The fundamental issue to be resolved involves how to adjust F when only PK is known.

Method 2 is based on the assumption that by merely knowing the species differences in PK, without any knowledge of species differences in PD, it is not possible to make any adjustment in F from the value used in the extrapolation based on the applied dose basis. Put in other terms, knowing about pharmacokinetic differences in species still does not necessarily mean that equivalent doses in different species would yield the same response in different species. For example, does 1 ug of toxin per liter of mouse liver yield the same number of detrimental events per organ as would 1 ug of toxin per liter of human liver? Method 2 takes the position that information is not yet available to answer that question (pharmacodynamics); thus, some value for F must still be applied. Further, it is not possible to discern how much of F is due to pharmacokinetic differences and how much is due to pharmacodynamic differences. Physiologically based pharmacokinetic models are not able to account for pharmacodynamic differences.

Figure 1 is a diagrammatic simplification of extrapolation between species. The horizontal arrow between applied doses represents the extrapolation factor F, which has been traditionally applied prior to incorporating pharmacokinetic data. The three horizontal dotted arrows represent interspecies correlations for the pharmacokinetic and some of the pharmacodynamic factors. Assuming that the pharmacokinetic factors are understood for a particular case, one is left with the problem of assigning factors to account for interspecies

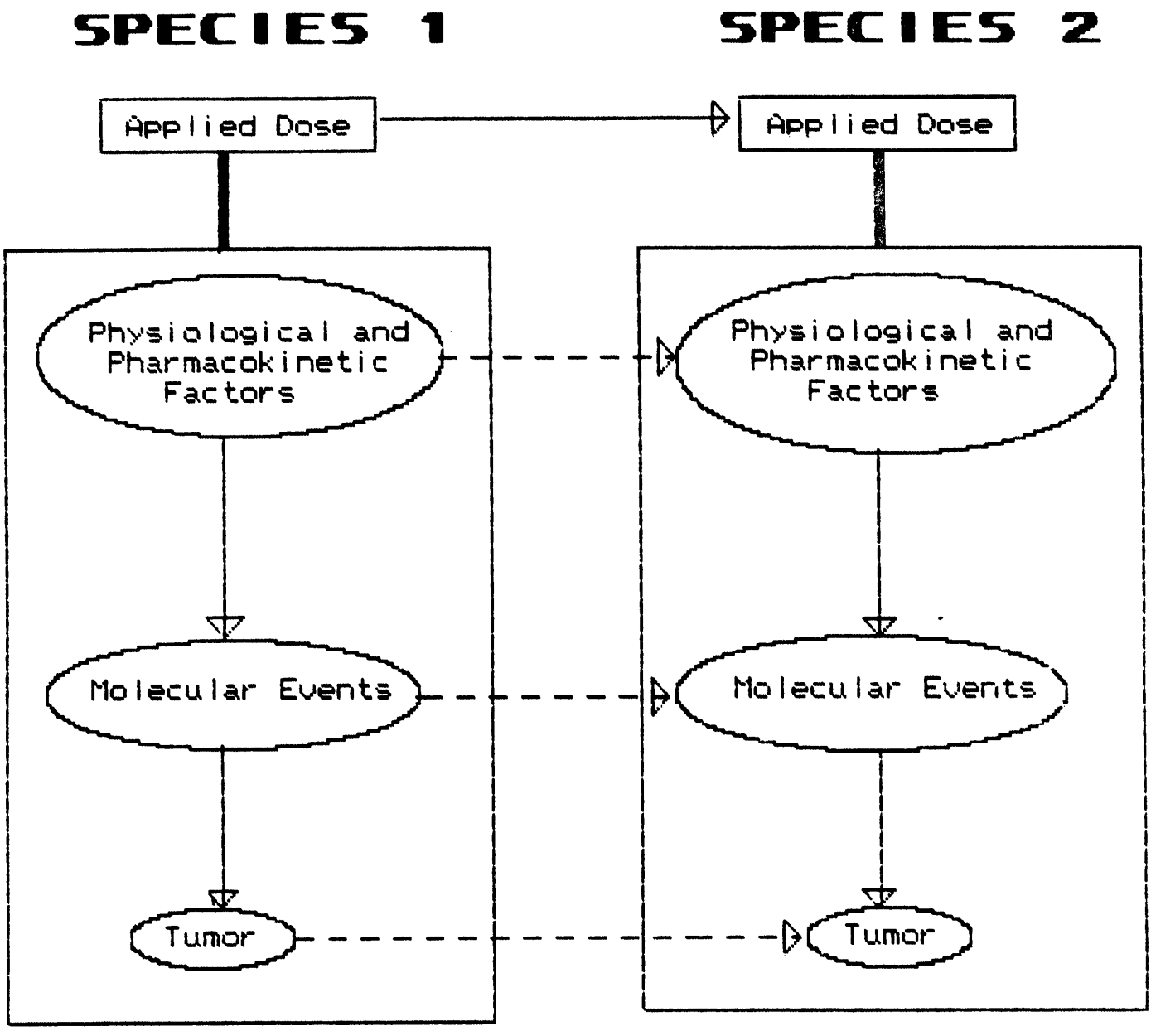

Figure 1. Extrapolation between species.

differences in the pharmacodynamics. Little information currently exists from which accurate estimates of these factors could be calculated.

At present there is some preliminary empirical evidence to suggest that applied dose factors (F) used by the various federal regulatory agencies are not in great error. However, it is not at all clear that those factors can be applied to account for pharmacodynamic events without some quantitative and perhaps qualitative modification. Method 2 implicitly assumes that the present data do not allow for determination of that adjustment.

How then can pharmacokinetic information be used if not for interspecies correlations? After reviewing the data and models with regard to DCM, it became apparent that there were pharmacokinetic differences related to differences in physiology and metabolism of the different species. It was also apparent that there were differences in the pharmacokinetics of high-dose exposure as compared with those of low-dose exposure. Method 2 accounts for differences in the high- to low-dose extrapolation within a species. However, for reasons described in previous paragraphs, interspecies differences in pharmacokinetics are not directly taken into account. The method is more thoroughly described by the HRAC (1987, Chapter 7), but briefly is accomplished as follows: Because it is not deemed possible to discern the true value of PD once PK is accounted for, the interspecies extrapolation is done by using F, that is, on an applied dose basis at the NTP dose. The physiologically based

pharmacokinetic model is then used to extrapolate from the high dose in the human to the low dose at which the unit risk is calculated. When this is performed, the effect of accounting for the nonlinearity in the pharmacokinetics from high to low dose results in a reduction of risk from the applied-dose method. The reduction is 2.1-fold for the lung and 4.4-fold for the liver. This reduction is the same regardless of what basis of F was used (Chapter 7 of the HRAC report provides a detailed derivation of these numbers). The sensitivity analyses (HRAC, 1987, Chapter 7) indicate that the 2.1-fold reduction is a minimum reduction that appears to be necessary, and so it is used as a basis for altering the low-dose human risk estimate. The resulting estimated human risk from continuous lifetime exposure to 1 ug/m^3 is 1.8×10^{-6}. This is 2.1-fold below the EPA estimate based on applied dose of 4.1×10^{-6}.

When extrapolations are performed, several assumptions are made. The examination of these assumptions and their implications on the risk assessment process is as critical as the estimation procedures themselves. In Method 2, some assumptions are generic in nature and others apply to the present case of DCM exposure.

First, this method assumes that at present it is not possible to discern if a unit of toxin per unit of mouse tissue would result in the same response as an equal unit of toxin per same unit of human tissue. As previously stated, this method assumes that the interspecies correlation factor used, results

from interspecies differences in both pharmacokinetics and pharmacodynamics. At present for DCM, this method assumes that the data are insufficient to determine how much of the correlation factor is due to differences in pharmacodynamics. Thus, it does not apply the interspecies correlation factor only to pharmacodynamics but to applied dose. A disadvantage of this assumption is that interspecies differences in pharmacokinetics, which can be accounted for quantitatively by modeling, are not weighed in the extrapolation procedure.

Second, this method actually quantifies pharmacokinetic differences that result in nonlinearities between high and low doses. As a result, for this case, the method appears to be somewhat insensitive to variations in the linear metabolic term, the first-order rate constant for metabolism by the GST pathway. This is both advantageous and disadvantageous. As described in subsequent chapters, the rate constant associated with the GST pathway is one of the most uncertain parameters in the physiologically based pharmacokinetic model. Because of the approach's insensitivity to this parameter, any errors do not greatly affect (within certain limits) the risk estimation. However, this insensitivity means that the approach will not reflect the impact of any new data that may be generated which show that GST activity in humans is significantly different than the present estimates.

Third, because of the nature of the pharmacokinetics of DCM as described by the pharmacokinetic model (Andersen et al., 1986,

1987), the reduction of 2.1-fold for lung and 4.4-fold for liver would also apply if the parent compound contributes to carcinogenicity.

Fourth, the method depends on several assumptions regarding the relative roles of the two metabolic pathways to carcinogenicity. The modification of the risk number calculated assumes the products of the MFO pathway to be of less importance than the products of the GST pathway in the possible mechanism of carcinogenicity. Although the evidence for this assumption, as discussed in the HRAC (1987) report, is strong, some doubt does linger.

Fifth, the model used by Andersen et al. (1986, 1987) partitions metabolism between lung and liver for both pathways. The methods used to determine the relative apportioning of metabolism between the two organs and the sensitivity of the model to those parameters are discussed in the HRAC (1987) report. The parameters used in the model for the estimation in this method are those used by Andersen et al. (1986, 1987) and are subject to uncertainty. The impact of any error in those parameters on the risk estimation could be significant.

Sixth, the method assumes that the structure of the model is correct. This implies that carbon dioxide observed at low doses arises from the MFO pathway. If true, this would be consistent with the theory that the GST pathway is not operational at low doses. The HRAC (1987) report discusses in great detail the uncertainties with this assumption. Obviously, if this

assumption were incorrect, one interpretation could be that the GST pathway is operational at low doses, and thus the model's structure would have to be significantly modified.

In summary, this method assumes that most of the parameters used in the model of Andersen et al. (1986, 1987) are within reasonable limits (with some adjustment for breathing rates). It assumes that the GST pathway is the predominant path to carcinogenicity. However, even if there is a nonsaturable portion to the MFO pathway (e.g., leading to carbon dioxide) which has a role, or if the parent compound has a role, the effect on these would be similar in concept as described above for the GST pathway (i.e., the effects of a saturable system regarding high- to low-dose differences on other nonsaturable systems). Again, it should be remembered that some or all of the intermediates of the various pathways, or the parent compound, may contribute to the carcinogenic process. However, if the estimates of partitioning of metabolism between liver and lung are incorrect, then the method could be in error. Further, this method makes no assumption regarding equivalency of delivered doses between species. It applies interspecies correlation factors in a manner that do not require knowledge regarding how much of the difference between species response is due to pharmacokinetics and how much is due to pharmacodynamics.

8.3. COMPARISON OF METHODS 1 AND 2

As with any risk estimation that involves extrapolation, many assumptions affect the actual risk numbers, and Methods 1 and 2 are certainly no exception. Both use pharmacokinetic information in an attempt to reduce uncertainties inherent in risk assessments. It is commonly, although erroneously, conceived that by incorporating pharmacokinetic information into a risk assessment, magical reductions of uncertainity are achieved. Actually, the examination and application of pharmacokinetic data and models for this compound have revealed something quite different. As one becomes more familiar with developing and using this type of information, new and often more complex questions arise. The utility of pharmacokinetics is, in fact, this very point. It allows for a systematic analysis of a chemical's disposition in the body, an important component of the risk assessment. In applied-dose approaches, assumptions are frequently made which, although sometimes based on empirical evidence, are often inflexible and thus in error at some conditions of the human exposure. For example, absorption fraction is frequently set at some arbitrary value determined from some empirical evidence or from assuming the "worst case" of 100% absorption. Pharmacokinetic modeling, when properly performed, is able to account more logically and realistically for amounts absorped on a time basis. Pharmacokinetic models seek to account for instantaneous concentrations and changes in those concentrations that are due not only to changes in exposure

conditions, but changes in the physiologic responses as well.

As observed in earlier discussions and in the HRAC (1987) report, while many uncertainties are reduced, several of the "old problems" remain, and in fact, new challenges arise. Only with continued work and trial applications will the science continue to mature. Only two possible methods have been applied here, but given the body of evidence and the development of the science, at this time these two possibilities are considered the most reasonable.

Methods 1 and 2 employ many of the same assumptions, and yet vary in some very significant ways. Although the actual calculated numbers are almost identical (within a factor of 4 for liver and much less for lung), the methodologies are quite different. When in error even some of the common assumptions have different implications depending on the method chosen. However, the differences are mainly in the "last step" of the risk assessment process, that is, how to actually use delivered dose to calculate a risk number.

A major and fundamental assumption that EPA has made for both methods is that the physiologically based pharmacokinetic model used by Andersen et al. (1986, 1987) is a reasonable method for describing and predicting the disposition of DCM and its metabolites in human tissues. This would include acceptance of the model's structure and input parameters. The HRAC (1987) report raises several important questions that are deemed important, and future elucidation for purposes of methodology

development will be necessary. However, for the present, EPA has applied the model with some minor changes. It was felt that the uncertainity raised by questions regarding model structure were no greater than those raised by a conventional applied-dose risk assessment. In fact, because the model is able to quantitatively describe numerous physiologic and biochemical processes, it is highly probable that model structure questions pose less uncertainty than the traditional approach. The HRAC is less certain about some of the input parameters, such as the metabolic rate constants. The consequences of errors in these could be great, and the impact may be somewhat different depending on whether Method 1 or Method 2 is employed.

There are three major sources of uncertainity with the metabolic scheme and parameters in the model. First, the model assumes that any carbon dioxide observed at low doses is being produced from the MFO pathway. The implication is that the carbon dioxide observed by several investigators at low doses is still compatible with the assumption built into the model that the GST pathway is virtually inactive at low doses. If incorrect it would mean that the GST pathway is active at low doses, where the model is predicting that it is not. Both methods would be in significant error in predicting risk at low doses (underprediction).

A second uncertainity common to both Methods 1 and 2 are the values of the input parameters which apportion metabolism by both pathways between the liver and lung. As discussed in the HRAC

(1987) report, there is great concern over the values estimated for these parameters. The pharmacokinetic model is quite sensitive to these parameters, and thus any error would be reflected in model predictions. Such error would be significant in both methods (over- or underprediction).

The third uncertainty regarding metabolism is with the values of the metabolic rate constants. As discussed in the HRAC (1987) report, most questions remain with the value determined for the first-order rate constant for the GST-mediated pathway. Determined by allometeric scaling and "curve fitting" of the model to exposure chamber data, the value of this parameter is uncertain. In fact, data from CEFIC (1987b) indicate that the value selected by Andersen et al. (1986, 1987) is in error. Significant questions also remain regarding the methodology and results of the CEFIC experiments. It is reported that experiments are being conducted which may reduce some of the uncertainty with regard to this rate constant. Method 1 is more sensitive to this parameter than is Method 2; thus, any error would result in greater error in the risk number calculated using Method 1. However, because of this sensitivity, if the value of this parameter is established more accurately, Method 1 would better reflect the impact of such findings.

As described previously, numerous uncertainties are associated with the assumptions that have been made to develop a risk assessment using pharmacokinetic information for DCM. Apart from the major generic difference between Methods 1 and 2, there

is also the uncertainty associated with the metabolic rate constant of the GST-mediated metabolic pathway. The risk calculation resulting from Method 1 would be greatly lowered by significant reductions in the estimate of this parameter.

Also, one might want to allow for some possible minor role of metabolism by the MFO-mediated pathway in the carcinogenic process. No current evidence suggests a contribution by this pathway, and a large role is ruled out by the very low tumor response in the National Coffee Association drinking water bioassay (NCA, 1982a, b; 1983), in which the MFO metabolism was saturated at a level similar to those associated with the highly tumorogenic exposures in the NTP inhalation bioassay (NTP, 1985, 1986). However, even a small contibution to DCM's carcinogenicity by MFO metabolism at high doses might have a meaningful impact on low-dose risks, since the proportion of the dose metabolized by this pathway increases at low exposure levels. One might, for example, hypothesize that 5% of the carcinogenic risk to mice at the bioassay doses resulted from MFO metabolism. (Any higher contribution begins to conflict with the observed lack of correlation of MFO metabolism--and clear correlation of GST metabolism--with tumor incidences.) Such a small contribution to tumor production by MFO metabolites would not have a major impact on the human risk estimates as they have currently been calculated using Method 1. If, however, the human GST metabolic rate constant were greatly reduced from the present estimate, resulting in much lower predicted human risk from this

pathway, that same hypothetical 5% contribution from MFO metabolism would have a far greater impact on the total human risk estimate at low doses. Neglecting a contribution of as little as 5% by the MFO-mediated pathway towards carcinogenesis would, under those circumstances, greatly underestimate the risk. If new data indicate that human GST activity towards DCM is much less than the estimate used here, then a reevaluation of the assumptions would be necessary. More confidence in the assumption that the MFO path does not contribute to carcinogenicity and greater certainty in the values of the appropriate metabolic parameters will be required before the concomitant reduction in the risk estimates would be accepted as appropriate.

Another question that arises, regardless of method, is upon which organs are risk estimates to be based? The pharmacokinetic approach gives information regarding specific organs. Site concordance of tumor production between animals and humans is not normally assumed in performing risk estimates. It is not clear how to extrapolate for the entire human (all organs) when risks have been calculated for specific organs by using pharmacokinetic knowledge. One possible solution is to select the organ with the highest risk number and apply this to the whole body. This could result in an overestimation of the risk for many organs but would ensure that no underestimation would occur due to a lack of knowledge about an oversensitive or a highly metabolic tissue. Alternately, if individual organ risks occur independently, they

could be mathematically summed. Both Methods 1 and 2 could do either of these. However, in the case of DCM, because of the comparative insensitivity of Method 2 to the GST metabolic parameter, even organs with several fold greater metabolic activity than the lung would not be expected to have a risk far different from that calculated here. The results of Method 1, however, are more difficult to apply to other organs. A several fold change in the GST level (as might be observed in other organs) would result in a different value for the risk number. Without knowing specific GST activities towards DCM in other tissues, it is difficult to ascertain the impact of such an uncertainty. Although there is no clear evidence of carcinogenicity in organs other than the lung or liver, there are some findings that raise concern about this issue. Benign mammary gland adenomas and salivary gland tumors developed in rats (NTP, 1985, 1986). The HRAC (1987, Chapter 6) discussed pancreatic tumors in workers exposed to DCM. Although these tumors may not be significant, some note should be taken.

It is clear that, once estimates or measurements of internal dose at the sites of toxic action are obtained, many difficult issues must be resolved as to how to use such data in the extrapolation of risk from experimental animals to humans. The problem is not confined to DCM, nor does it result from any shortcoming in the information on the pharmacokinetics of this compound. It is a general problem, reflecting the lack of understanding of the pharmacodynamics of carcinogenesis.

As discussed earlier, there are many difficulties in using metabolic differences in species to modify a carcinogenic risk assessment. Extrapolation between species involves many factors, including metabolism and pharmacokinetics. The ability to elucidate a species difference in one contributing component does not necessarily indicate what, if any, adjustments should be made to the overall extrapolation. It does not necessarily provide more certainty than the empirical process currently used; in fact, making the necessary assumption may introduce new questions.

Method 1 advocates the adjustment of the applied-dose risk extrapolation by the degree to which humans (at lower doses) and the bioassay rodents metabolize different proportions of their applied doses at the internal site of carcinogenic action. In the present case, this method leads to a risk reduction of 8.8-fold from the level estimated in EPA's previous applied-dose risk assessment. In Method 1, the observed pharmacokinetic differences between species are to be compared with those expected to emerge as a result of differences in physical size and the rates of physiological processes in rodents and humans.

Method 2, which leads to a risk reduction of 2.1-fold, advocates the adjustment of the applied-dose risk extrapolation only by the degree to which the proportion of the applied dose that is metabolized differs from high human doses to low human doses; any species difference in the proportion of the dose that is metabolized is ignored as a basis for determining human

carcinogenic potency. Instead, the interspecies component of extrapolation is carried out as would be done if using applied dose. The reasoning is that, in addition to the effect of species differences in metabolism, there are expected (but unknown) differences in the carcinogenic responsiveness of the tissues to a given delivered dose. Pharmacokinetic data illuminate only the metabolic differences. It may be, for example, that greater sensitivity to carcinogens in humans "compensates" for lower metabolic activation of the applied dose. The justification for using the surface area correction on the applied dose during the species-to-species extrapolation rests on tradition. Empirical comparison of carcinogenic potencies in humans (determined directly from epidemiologic data) with those from experimental animals shows the surface area scaling relationship to be a reasonable estimator for many compounds, although other chemicals show potencies that differ from the expectation based on this relationship by orders of magnitude.

Because of the problem of specifying interspecies differences in tissue responsiveness to carcinogens, both Methods 1 and 2 can only give a relative adjustment to the applied-dose calculation of human risk. That is, incorporation of pharmacokinetic information can only raise or lower the "dose delivery" component of interspecies extrapolation relative to its appearance in the applied-dose procedure, while the component representing "responsiveness" or pharmacodynamic differences between experimental animals and humans remains problematic and

continues to be based on assumptions retained from the former applied-dose procedure. Methods 1 and 2 differ chiefly in the way that assumptions from the applied-dose extrapolation procedure are retained when data on the pharmacokinetic component are available.

Method 1 is based on the conclusion that, given the most reasonable scaling of key physiological variables across species, delivered dose is expected, a priori, to be the same proportion of applied dose in rodents and in humans. That is, differences in body size and physiological rates between rodents and humans do not, in themselves, lead to an expectation of differences in the delivered doses of metabolically activated carcinogens. If the proportion of a dose that is metabolized is in fact the same across species, then the applied dose serves as a good surrogate measure for the delivered or internal dose of a carcinogen, and both dose measures will result in the same risk extrapolation. Thus, the surface area correction, as traditionally used in the applied-dose procedure at EPA, corresponds to an assumption about (and correction for) interspecies sensitivity differences rather than about metabolic differences. It is the factor by which human risk is assumed to exceed mouse risk for a given dose (applied or internal). This same assumption about relative sensitivity is retained when a pharmacokinetic analysis of the proportion of a dose that is metabolized replaces the prior assumption of equality of dose delivery across species, implicit in the use of applied dose in extrapolation.

In the present case, for example, the surface area correction between mouse and human doses is a factor of 12.7. According to the model used by Andersen and Reitz, the species difference in metabolism is such that humans (at low exposure levels) metabolize about one-ninth as much of their applied dose via the GST pathway as do mice at 2000 or 4000 ppm. (Most of this difference is due to high- to low-dose differences that result from the saturation of the competing MFO pathway at the high bioassay exposures experienced by mice--the interspecies difference at the same applied dose is quite small.) According to Method 1, the lower metabolic activation of DCM in humans implies that the carcinogenic potency difference between humans and mice is only one-ninth as large as it was previously thought to be, before the metabolism data were available. The carcinogenic potency in humans (expressed in units of applied dose) is only one-ninth as large as the value based on applied dose.

Method 2, in contrast, suggests that no reasonable assumption can be made about the effect of allometric scale on metabolic differences among species. Under this view, any magnitude of species difference in metabolism seems equally probable a priori, and so there is no prior assumption against which to compare empirical data on the actual difference. Instead, it is presumed that, for a given dose level, the combined effect of metabolic and sensitivity differences is given by the surface area correction on applied dose. No explicit

assumption about the species difference in sensitivity is made; in fixing the magnitude of the combined effect, however, a value of the sensitivity component is assumed implicitly. For example, in the present case the pharmacokinetic model estimates that humans at high doses metabolize 4.5-fold less of their delivered dose in the lung and 1.5-fold less in the liver than do mice at equally high doses. By assuming that the overall interspecies factor is 12.7, Method 2 implicitly assumes that these metabolic deficits are compensated for by greater human sensitivity of 57.2-fold in lung (1/4.5 x 57.2 = 12.7) and 19.1-fold in liver (1/1.5 x 19.1 = 12.7). Low-dose human risks are adjusted by the degree to which the proportion of the applied dose that is metabolized via the GST pathway is different than at these high human doses. That is, delivered dose is used only for the extrapolation within species, where the question of interspecies difference in sensitivity does not arise.

Thus, the crux of the difference between the two methods is whether or not a reasonable prior assumption about the expected species differences in metabolism can be made before pharmacokinetic data are available. If a prior expectation can be specified, when pharmacokinetic data become available, one may replace that assumption with data (which may show the assumption to have been inappropriate for that compound). The same assumption about species differences in sensitivity is applied in all cases. If, on the other hand, no prior expectation about pharmacokinetic differences between species can be specified,

there is no way to know whether the observed differences are bigger or smaller than usual. The applied dose is therefore used to extrapolate across species, and the sensitivity assumption is adjusted to make its combined effect with the observed metabolic differences come out to be equal to the surface area correction, since it is assumed that the combined effect scales in this way.

The choice between Method 1 and Method 2 has not been an easy one, and has been made only after considerable debate and discussion both within EPA and with representatives of other federal regulatory agencies. The attributes of each method that have been considered include their relative conservatism in the face of uncertainty, their sensitivity to errors in the underlying assumptions and estimates of the pharmacokinetic model used by Andersen and Reitz, their correspondence to previous practice, their ability to incorporate current understanding of metabolism, however imperfect, into the risk extrapolation process, and, of course, the plausibility of the assumptions upon which they are founded.

EPA concludes that Method 1, which extrapolates risk across species and from high to low doses based on the amount of metabolism of DCM by the GST pathway, is the most advisable basis for use of current pharmacokinetic information. The evident importance of differences in metabolism among rats, mice, and hamsters to DCM's carcinogenic potency in these species makes the use of metabolic differences desirable in the estimation of human

risk. While acknowledging that many factors in addition to pharmacokinetics influence species differences in carcinogenic potency, EPA concludes that it is reasonable to modify risk extrapolation from experimental animals to humans by the degree to which the species manifest different degrees of metabolic activation of their applied doses at the site of carcinogenic action. The absolute levels of human risk that are estimated remain uncertain, as always, owing to the lack of knowledge about the contributions of the other, non-pharmacokinetic factors to the relative carcinogenicity of DCM in rodents and humans. The need to retain assumptions about the role of such factors should not, in EPA's opinion, dissuade us from examining the potential contribution of such factors as can be experimentally examined. The choice of Method 1, the choice of the GST pathway as the sole route to carcinogenic activation, and the choice of the model used by Andersen and Reitz as a means of its estimation have been made because, in EPA's judgment, they represent the most likely and plausible interpretation of the data at hand. Each choice is made in the face of some uncertainty, and the interpretation of the resulting estimate of the carcinogenic potency of DCM in humans must be tempered with the knowledge that further data may lead to other choices and different risk estimates becoming more defensible.

The unit risk for continuous inhalation of 1 ug/m^3 of DCM is thus estimated as 4.7×10^{-7}. For comparison, the applied-dose extrapolation leads to a value of 4.1×10^{-6} (which is 8.8 times

higher), and the use of the same metabolic data, but extrapolating to human risk using Method 2, results in a value of 1.8×10^{-6} (which is 2.1-fold lower than the applied-dose method and 3.8-fold higher than Method 1).

It should also be noted that both Methods 1 and 2 are presented as modifications of the method of risk extrapolation to humans commonly employed by the EPA and CPSC, that is, with the surface area correction used as an interspecies correction factor. If one instead uses the body weight basis for defining equally risky applied doses in animals and humans (as is done by the FDA), then the estimated human risk by all methods would be 12.7 times lower. That is, the applied dose procedure would lead to a unit risk estimate of 3.2×10^{-7}, while modifications of this unit risk by accounting for metabolism would yield unit risks of 3.7×10^{-8} for Method 1 and 1.4×10^{-7} for Method 2. These numbers are 12.7-fold, $8.8 \times 12.7 = 111$-fold, and $2.1 \times 12.7 = 26.7$-fold, respectively, below the published EPA unit risk of 4.1×10^{-6} based on applied doses scaled by surface area. Andersen et al. (1986, 1987) and Reitz et al. (1986) argue that, because interspecies differences in metabolic and physiologic parameters have been accounted for by the pharmacokinetic model, there is no longer a need for any interspecies correlation factor. Implicit in this view is the assumption that the interspecies correction factor on applied dose is used solely to account for species differences in metabolism, and that metabolic differences completely account for differences in carcinogenic

potency of a compound in animals and humans. The EPA takes the view that this is not the case, since it ignores the contribution of non-pharmacokinetic factors that influence a species' responsiveness to a given internal dose.

Lifetime extra risks over background from continuous and constant low-level exposure to DCM may be estimated by multiplying the vapor concentration by the internal unit risk value. However, the EPA's analyses of the model used by Andersen and Reitz indicate that, if vapor concentrations exceed 100 ppm or so for any part of an exposure, substantial nonlinearities begin to appear that tend to invalidate the assumptions allowing the unit risk to be used. Under such conditions the MFO pathway begins to show saturation, resulting in disproportionally more DCM being available to GST metabolism, which results in disproportional increases in internal dose. Exposures involving high vapor concentrations can have estimated risks that are several fold above the levels implied by the "equivalent" time-weighted average exposure. The reader is also reminded that the unit risk assumes a breathing rate of 20 m^3/day. Occupational exposures, or other exposures occurring during more-strenuous-than-average activity, will consequently have risks somewhat underestimated.

Although EPA feels that it is warranted to use species-to-species pharmacokinetic and metabolic information to adjust estimates of human risk based on animal data, the absolute levels of estimated human risk remain uncertain, owing to the unknown

contribution of species differences in sensitivity to a given internal dose of carcinogen. EPA recommends that intensive efforts be made to develop information on the pharmacodynamics of carcinogenesis that could be used in the risk assessment process in the future. One approach, which may elucidate the magnitude and variability of the pharmacodynamic factor for various species comparisons, is to obtain pharmacokinetic information in both animals and humans for known human carcinogens. This would allow an implicit determination of the pharmacodynamics for humans relative to various rodent species, since the contribution of pharmacokinetics and the relative potencies of applied doses could be estimated from available data.

9. IMPACT OF CEFIC EXPERIMENTAL DATA ON RISK ESTIMATES

The following analysis provides an indication of the changes in estimated risk implied by the results of CEFIC's (1986e) in vitro metabolism experiments. These studies found no detectable activity of human liver cytosol toward DCM. The authors raised the possibility that humans do not have the specific isozyme(s) of GST that are active on DCM. Recently, however, scientists from CEFIC and Dow Chemical Company have reported that they have each independently detected low GST activity in human tissues using a more sensitive assay. (This information was reported by Green and Reitz in a May 1987 letter to EPA). No results have been made available to the federal regulatory agencies at this time, however. The following analysis is, therefore, based on the limit of detection of the original CEFIC (1986e) work.

If the human GST isozyme(s) does act on DCM, it is no more than one-sixtieth as active as the mouse GST system (expressed as nmoles/min/mg). This assumption is based on the limit-of-detection reported by CEFIC for their assay, i.e., the mouse value of approximately 36 nmoles/min/mg divided by the limit-of-detection, 0.6 nmoles/min/mg. Determining the impact of such a level is extremely complex and requires a number of additional assumptions in order to derive an idea of the in vivo effect on risk of assuming that human GST is one-sixtieth as active as mouse GST on DCM in vitro. By way of example, one set of assumptions is adopted below to give an idea of the potential

effect on risk. Alternative assumptions, which would give different results, are still under consideration by the HRAC (1987).

The CEFIC data on in vitro GST metabolism by the human liver cell fractions show no detectable activity toward DCM (four livers were tested). On the basis of several assumptions, the limit-of-detection of GST activity reported in the CEFIC study can be used to set an upper limit on the parameter describing the rate of human GST metabolism (k_F) in the pharmacokinetic model used by Andersen and Reitz. The CEFIC analysis treats the GST pathway as saturable at high substrate concentrations (higher than experienced in vivo), and hence represented by Michaelis-Menton kinetics. In determining an upper limit on human GST metabolism, the limit-of-detection CEFIC reported (0.6nmol/min/mg protein) can be regarded as the maximum rate attainable by the human GST pathway (V_{max}). This rate is 60-fold lower than the maximum in vitro rate CEFIC observed for mouse liver (36 nmol/min/mg protein)

At lower doses (well below saturation) the rate becomes directly proportional to the DCM concentration and thus directly comparable to the rate in the model used by Andersen and Reitz. The single rate constant used by Andersen et al. (1986, 1987) to represent GST metabolism (k_F) is, at low doses, essentially equal to CEFIC's maximum rate (V_{max}) divided by the Michaelis constant (K_M). This constant reflects the interaction of the GST enzyme with its substrate. The human value of K_M cannot be estimated

from the limit-of-detection, but if it is assumed that human K_M is equal to the CEFIC estimate of K_M for mice, the GST pathway in vitro rate constants (k_F's) for mice and humans will be in the same ratio as the V_{max}'s; that is, the human value will be 60-fold lower than the mouse value.

Extrapolating from such in vitro estimates to the expected rate of metabolism by a whole intact organ is also problematic. If it is assumed that the mouse/human ratio of k_F's in vivo is the same as the ratio of the in vitro estimates developed above, then the human whole-liver k_F should be one-sixtieth that of the mouse, or $4.30/60 = 0.072\ hr^{-1}$. This value can then be compared to the value of $0.53\ hr^{-1}$ in the model used by Andersen and Reitz. In other words, if the human GST metabolism constant in the model used by Andersen and Reitz is replaced by one estimated from the limit-of-detection of the CEFIC in vitro data, the rate of human GST metabolism predicted by the modified model is decreased by about sevenfold, assuming the Andersen and Reitz value for mouse k_F is correct.

To use this result in risk estimation, the assumption must be made that GST metabolism in human lung is reduced by the same amount as that for the liver. At present, no human in vitro data on human lung are available to make this estimate directly. Furthermore, it is only a rough approximation to say that a sevenfold change in the model's parameter describing GST will lead to risk estimates sevenfold lower. Revising the GST kinetic parameter in the pharmacokinetic model used by Andersen and Reitz

is a major change which should involve reconsideration of other parameters, particularly those representing MFO metabolism. Parameter adjustment reopens the issue of model validation. A constructed model could give results indicative of something quite different from a sevenfold reduction in risk.

In summary, an analysis of the implications of the results of the CEFIC in vitro metabolism experiments, suggests that risks from exposure to DCM could be some sevenfold lower than estimates from the unmodified model used by Andersen and Reitz, based on a rough estimation procedure. Green and Reitz reported, in a May 1987 letter to EPA, preliminary results of new studies that use ^{36}Cl-DCM; these results should greatly increase the level of detection of GST activity. Furthermore, the CEFIC experiments designed to provide new values for several model parameters, including partition coefficients and kinetic parameters, may lead to a modified pharmacokinetic model that is better validated and, thus, could more confidently be used in estimating human risks.

10. IMPACT OF EPIDEMIOLOGIC EVIDENCE ON HUMAN RISK ESTIMATION

Two historical cohort studies examine the mortality experience of workers occupationally exposed to DCM; one evaluates triacetate fiber extrusion workers (Ott et al., 1983) and the other, Kodak film-casting workers (Friedlander et al., 1978; Hearne and Friedlander, 1981). Neither study reports a statistically significant increase in deaths from cancer among workers exposed to DCM. The Kodak study, however, which is the more powerful of the two studies, with far better exposure information, was used for EPA's quantitative analysis. This study has recently been updated by the addition of 262 men, an increase of up to 8 years in the length of follow-up on the original cohort members, and an investigation of the possible contribution of potential confounding factors, such as smoking (Hearne et al., 1986).

The updated Kodak study examines a 1964-1970 cohort of 1013 male film-casting employees evaluated through 1984. Time-weighted exposures over the duration of the workday averaged 26 ppm, and ranged from 20 to 140 ppm, depending on job category. Exposure duration averaged 22 years. Extensive monitoring and detailed job history information permitted the calculation of individual exposure histories. The mortality experience of these workers was compared against two sets of standard rates: New York State males and nonexposed male hourly employees at Kodak's Rochester facilities. The second comparison is more informative,

as it largely eliminates the "healthy worker effect."

The results show no elevation of total deaths from malignant neoplasms (41 observed versus 52.7 expected), nor of respiratory cancer deaths (14 versus 16.6), nor of liver cancer deaths (0 versus 0.5). Neither was there a trend in incidence of respiratory cancer deaths with increasing exposure or with increasing time-since-first-exposure. Hearne et al. (1986) state that no other cause of death was statistically elevated, but noted a nonsignificant excess of pancreatic cancer deaths (8 versus 3.1 expected) which they attribute to expected statistical fluctuation when several end points are examined simultaneously.

Hearne et al. (1986) interpret their study as showing that DCM is safe for humans at the occupational exposure levels experienced in the study. They have calculated the number of excess cancers that would have been expected in the cohort if the EPA's published incremental cancer risk (U.S. EPA, 1985b) were true, and claim greater than 99% power to detect such an elevation if it were manifested as lung cancer deaths. Hearne et al. concluded from their analysis that the federal regulatory agencies have overestimated DCM cancer risks to humans.

Two reviews of the updated Kodak study, an EPA evaluation (HRAC, 1987) and an independent analysis by Dr. G. Matanowski (Batelle, 1986), found fault with some aspects of the comparison of exposed workers to nonexposed workers. Both reviews also criticize the study on the grounds that the Kodak cohort includes a large number of men with extensive exposure before the

enrollment date; these men thus represent survivors of previous exposure, and the sample could be biased away from sensitive individuals who may have left their film-casting jobs due to illness.

Despite the criticisms of the Kodak study, EPA finds it to be generally well conducted, but the study's apparent disagreement with the carcinogenic effects of DCM as observed in the NTP bioassay must be analyzed. A CPSC review (Cohn and Rock, 1986) points out that the pancreatic tumors observed in the Kodak study are significantly elevated if a 5% level of significance is used rather the 1% level that Hearne et al. apply to "non-hypothesized" causes of death, i.e., causes not indicated by animal studies. EPA recognizes that the increase in pancreatic tumors may merely reflect the fact that a few apparent increases, even statistically significant increases, can be expected (even when no excess exists), due to chance alone. The increase in pancreatic tumors cannot be considered an unequivocal positive response and should not be interpreted as evidence that DCM is a human carcinogen. Nevertheless, an increase of this magnitude raises some concern about possible human response in tissues other than those found to respond in animals, a not uncommon phenomenon.

Quantitative analyses have been conducted to determine whether the results of the Kodak study, which show no statistical increase in cancer deaths with the exception of a marginally significant increase, at most, in deaths from pancreatic cancer,

refute the magnitude of risk estimated from the animal data. CPSC (Cohn and Rock, 1986) determined that CPSC's animal-based incremental risk estimate for DCM (which is slightly lower than the EPA applied dose estimate) predicts an excess of only 8.7 cancers in the Kodak cohort; such an excess of liver cancers could be detected, but the statistical power to detect 8.7 excess lung cancers is only 55%. CPSC notes that the observed excess of pancreatic tumors in the Kodak study of almost 5 deaths is of the same order as their prediction of 8.7 excess cancers, especially since the cohort is rather young, with only 18% having died from all causes, while the animal-based predictions are for lifetime risks.

EPA's analysis (HRAC, 1987) takes a somewhat different approach: the Kodak study is used to directly generate a human risk estimate, and a 95% upper bound to that estimate, which may then be compared to the animal-based upper-bound incremental risk estimates. This analysis uses age-specific data on cumulative DCM exposure and on observed cancer risk. Two models are applied. The first is an additive excess risk model, in which one assumes that the excess cause-age-specific death rate due to DCM exposure is increased in an additive way by an amount proportional to the cumulative exposure up to that age. The second is a multiplicative or relative risk model, in which the cause-and-age-specific background rate at any given age is increased by a multiplicative factor proportional to the cumulative dose up to that age. An adjustment for a latent

period of about 20 years is made by examining exposure and risk only after age 45.

When applied to the data on pancreatic cancer deaths in the Kodak study, the additive excess risk model procedure and the relative risk model procedure yield a maximum likelihood estimate of incremental risk from a lifetime exposure to 1 ug/m^3 of 3.4×10^{-6} and 1.4×10^{-6}, respectively. The 95% upper bounds on the Kodak-based incremental risks are 7.1×10^{-6} and 2.8×10^{-6}.

EPA applied the same analysis to respiratory cancers among Kodak workers. Since fewer cancers were observed among exposed workers than expected based on nonexposed workers, the best estimate of incremental risk is zero. The 95% upper bound on this incremental risk is 7.6×10^{-7} for a lifetime exposure to 1 ug/m^3 according to the multiplicative model. (The additive model could not be estimated for these data.)

The HRAC's (1987) calculation of an upper bound on DCM potency based on the Kodak respiratory deaths is about fivefold below the EPA applied dose extrapolation from mice; this result is compatible with the Hearne et al. conclusion that their study has over 99% statistical power to detect an increase in cancer in the Kodak cohort of the magnitude predicted by EPA's (U.S. EPA, 1985a, b) unit risk. When the Kodak pancreatic cancer deaths are used as the basis of human risk estimation, the maximum likelihood estimate of risk (3.4×10^{-6}) and the upper bound estimate (7.1×10^{-6}) are quite similar to the EPA applied dose value (4.1×10^{-6}). The Hearne et al. conclusion that the Kodak

results refute EPA's original risk estimates depends on the expectation of site concordance across species.

The issue of lack of compatibility of risks estimated from the NTP bioassay data and those made directly from human data is largely resolved when risks extrapolated from animal data are estimated from internal dose. Animal-based internal dose risk estimates, when extrapolated using Method 1, are 8.8-fold below EPA's applied-dose risk estimates and lower than all of the estimates made from the Kodak human data as well. The upper bound on carcinogenic potency based on the Kodak respiratory cancer deaths (7.6×10^{-7}) is more than twice as high as the lung-specific potency extrapolated by Method 1 from the internal dose in the NTP mice (3.3×10^{-7}). The maximum likelihood estimates of potency based on the Kodak pancreatic cancer deaths (3.4×10^{-6}) is higher than the combined liver and lung risks extrapolated by Method 1 from mice (4.7×10^{-7}). (If the pancreatic cancer deaths were to be taken as a clearly positive response in the Kodak study, which they are not, this fact would be grounds for using the human-based potency in preference to the lower animal-based estimate).

The human risk estimate that emerges by extrapolating from the NTP mice using Method 2 (1.8×10^{-6} per ug/m^3) is only 2.1-fold below the EPA applied-dose extrapolation. This animal-based risk estimate is of the same order as the maximum likelihood estimates based on the Kodak pancreatic cancer deaths (3.4×10^{-6}

and 1.4×10^{-6} for the additive and relative risk models, respectively). The 95% upper confidence limit on risk based on the Kodak respiratory cancer deaths (7.6×10^{-7}) is still some 2.1-fold below the animal-based risk when extrapolating by Method 2.

It can be argued that the Kodak study does not support the use of either pancreatic tumors or respiratory tumors to quantify risk, since these tumors were not clearly elevated to a statistically significant level. Lack of statistical significance does affect the qualitative weight of evidence regarding DCM's human cancer potential; as noted previously, the increase in pancreatic cancers cannot be interpreted as evidence that DCM causes cancer in humans. When looking at whether or not the Kodak study refutes the animal studies as a basis for human risk estimation, however, EPA believes it to be appropriate to compare quantitative estimates of cancer risks suggested either by tumors corresponding to sites found in the animal study (lung cancers) or by tumors with pronounced elevation.

Whether human risks are extrapolated from mice by Method 1 or by Method 2, the estimates are close to those derived directly from the Kodak study. Thus, the Kodak study does not contradict the conclusions that have been drawn from the animal studies. Because of the lack of positive responses, the epidemiologic studies do not add weight to the evidence of DCM's carcinogenicity in humans. However, these studies do not constitute evidence of its safety in humans either, nor do they

indicate that the animal-based risk estimates must be too high.

11. EPA'S CONCLUSIONS CONCERNING THE RISKS TO HUMANS FROM EXPOSURE TO DICHLOROMETHANE

Data from the 1985 NTP inhalation cancer bioassay demonstrate that DCM is oncogenic in two species of laboratory animals, rats and mice. In rats, tumors were benign fibroadenomas of the mammary gland. The literature (Russo et al., 1982) suggests that adenomas of this type are histogenically different from malignant adenocarcinomas and do not have a high potential for progressing to malignant tumors. As the rat tumors were not of a type with known malignant potential, the relevance of these tumors to human health is unclear. The response in rats cannot be entirely discounted but is considered to carry less weight than the response in mice.

In mice, the response was unequivocally carcinogenic. Administration of DCM via inhalation at doses of 2000 or 4000 ppm caused a statistically significant increase in malignant tumors in two organs, the liver and lung, in B6C3F1 mice. Although the tumors were of a type that have occurred at a high and/or variable background frequency in the strain of mouse tested, there is no question of their statistical significance in the NTP study. The tumor increases in mice were dramatic: at 4000 ppm 40 out of 50 male mice and 41 out of 48 female mice developed lung tumors; 33 out of 49 male mice and 40 out of 48 female mice developed liver tumors. Moreover, the tumors occurred in a pattern which meets the criteria for determining whether commonly

occurring tumors provide sufficient evidence of animal carcinogenicity; an excess of malignant tumors was observed in both sexes of mice, and the proportion of malignancies to benign tumors, as well as the time to first appearance of tumor, were dose related. The NTP data thus provide sufficient evidence of animal carcinogenicity.

The EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) and the Office of Science and Technology Policy (OSTP, 1985), generally accepted by the scientific community, recognize that chemical agents for which there are sufficient evidence of carcinogenicity in animals should be regarded as probable carcinogens for humans. The OSTP policy states that a "finding of carcinogenicity in rodents is proof that the chemical is carcinogenic in a mammalian species. Such a finding must be taken as strong evidence that the chemical can be a carcinogen in man, unless there is compelling. . .evidence to the contrary."

The varied response in chronic animal studies on DCM--a clear carcinogenic response in mice exposed at high doses, a negative response in hamsters, a primarily benign tumor response in rats, and the lack of a statistically significant increase in malignant tumors in mice exposed at low doses--are grounds for investigating whether there are biological differences between species that could lead to species (and/or dose related) differences in risk.

EPA has been concerned with evaluating whether the new data on DCM regarding species differences in metabolism and the

carcinogenic mechanism of action lead to the conclusion that DCM does not present a cancer risk to humans. EPA believes that to make such a statement, the data would have to indicate with reasonable certainty that:

1. the mechanism of carcinogenic action in mice is not expected to occur in humans; or
2. the carcinogenic biochemical pathway is inactive in humans; or
3. the epidemiologic data are sufficient to clearly indicate that DCM does not cause cancer in humans.

After a critical analysis of the evidence, EPA has concluded that the data fall short of meeting any of these criteria. The carcinogenic mechanism of action of DCM remains unidentified. There is little evidence supporting either an epigenetic or a genotoxic mechanism. Results from a host of genotoxicity studies are mixed. Unequivocal positive results were obtained chiefly in prokaryotic systems; positive results were fewer as the complexity of the test organism increased and test systems progressed from in vitro to in vivo. The insensitivity of the available mammalian in vivo studies and the lack of supporting evidence for an alternative mechanism allow the suspicion, however, that DCM may be a weak genotoxin in mammals.

Data on a possible cytotoxic mechanism are limited and difficult to interpret. Exposure to DCM brought on transient cytotoxic effects in specialized cells of the mouse lung, but the significance of this response to the induction of carcinogenicity

is unknown. Further, given that the human lung contains cells biochemically similar to the affected cells in the mouse lung, there is reason to believe that the cytotoxic response in mice could be repeated in humans.

Evidence of an epigenetic mechanism in the liver is still less certain. Data to support the hypothesis that the carcinogenic response in the mouse liver results from increased cell turnover are scant. An S-phase hepatocyte study registered small, variable increases in the incidence of S-phase cells after DCM exposure, but the authors of the study concluded that the biological significance of these changes is unclear.

Current evidence is simply not sufficient either to identify with reasonable certainty the mechanism of action of DCM or to indicate that said mechanism would not be expected in humans. Questions concerning the mechanism may be answered by additional research. CEFIC has underway studies to better define the role of the effects of DCM on the Clara cell in the development of mouse tumors. The results of this research are expected in the summer of 1987. NIEHS has planned investigations of the role of cell replication and the pattern of oncogene activation in DCM tumorigenesis. Preliminary results of NIEHS' experiments may be available in 1988.

It does seem likely from the available data that DCM produces a carcinogenic response via GST-mediated metabolism. The correlation between GST activity and the presence of tumors is strong, and there is little evidence to implicate either

parent DCM or the alternative MFO pathway. The level of GST metabolism in the mouse lung and liver has been shown to be high, but the degree to which humans metabolize DCM via the GST pathway is uncertain, at present.

The pharmacokinetic model used by Andersen and Reitz estimates human GST activity toward DCM to be not too far below the level in mice. The Andersen and Reitz values may be in conflict with CEFIC's estimates from in vitro measurements of GST activity in human liver samples, however. The CEFIC data indicate an absence of GST activity above the limit-of-detection of the test system. At present, it seems reasonable to assume that humans metabolize DCM via the GST pathway at a rate below that of the mouse, possibly far below. In fact, recent communications received from CEFIC and the Dow Chemical Company reveal that the preliminary results of in vitro studies using ^{36}Cl-DCM show that mouse liver has relatively greater GST activity towards DCM than does the liver of hamsters, rats, or humans. Rat and human liver tissues were judged to be the least active.

Neither do the epidemiologic data rule out a risk to humans, although the two studies of humans exposed to DCM in the workplace show no statistically significant increase in deaths from either liver or lung cancer. The study of Kodak film-casting workers, a particularly well-documented analysis, recorded no statistically significant excess of deaths from any type of cancer with the possible exception of pancreatic cancer. The Kodak cohort had an elevation in pancreatic cancer deaths

which is marginally significant if a 5% level of significance is used. The increase in pancreatic cancers is not considered an unequivocal positive response, but gives some weight to the possibility that a carcinogenic response in humans to DCM exposure could occur at sites others than those found to respond in animals. Further, a quantitative analysis of cancer risks estimated directly from the human data indicates that the results of the Kodak study do not refute the magnitude of risk estimated from the animal data, when animal-based risks are estimated on the basis of internal dose. The epidemiologic data are thus judged to be insufficient to clearly indicate that DCM does not cause cancer in humans.

EPA believes that the currently available data on mechanism of action, carcinogenic metabolic pathway, and epidemiology do not support a finding of zero cancer risk to humans from DCM exposure. If the risk to humans is not zero then what is the best estimate of risk?

Principle 26 of the OSTP (1985) states:

> No single mathematical procedure is recognized as the most appropriate for low-dose extrapolation in carcinogenesis. When relevant biological evidence on mechanism of action exists (e.g., pharmacokinetics, target organ dose), the models or procedures employed should be consistent with the evidence.

The OSTP principle is echoed by the EPA Guidelines which state that

> When pharmacokinetic or metabolism data are available, or when other substantial evidence on the mechanistic aspects of the carcinogenesis process exists, a low-dose extrapolation model other than the linearized multistage procedure might be considered more appro-

priate on biological grounds.

DCM has been shown to be metabolized in mice by two pathways--one of which, the MFO pathway, is saturated at high doses. Further, the second pathway, which is mediated by GST, appears to be less active in humans than it is in mice. Andersen et al. have developed a physiologically based pharmacokinetic model which provides a framework for estimating the nonlinearity inherent in metabolism by two pathways used to different extents at high and low doses, and, possibly, for incorporating species differences into the risk estimation procedure.

The pharmacokinetic model used by Andersen and Reitz is yet to be fully validated, and some of its parameters, in particular the partition coefficients and kinetic parameters, are considered to be uncertain. Further, there is debate within the scientific community over the best way to use the model's results in developing estimates of risk. Nonetheless, the pharmacokinetic model provides a means of taking into account metabolic data which would otherwise be ignored in the applied-dose procedure. Despite its uncertainties, the model allows the development of preliminary estimates of risk based on metabolized dose.

When the pharmacokinetic model is used with the kinetic parameters estimated by Andersen et al., it leads to a risk estimate that is 8.8-fold lower than that calculated under the applied-dose method, when extrapolation is done by a method called Method 1 herein. This estimate reflects the assumptions that the model can be used for both cross-species and cross-dose

extrapolation of internal dose. A somewhat different extrapolation method, referred to as Method 2, results in a lowering of risk of 2.1-fold. This method assumes that pharmacokinetics can at present be used only for high- to low-dose extrapolation, while species-to-species extrapolation is done on applied dose. Both methods assume that EPA breathing rate factors should be employed in the model used by Andersen et al. (1986, 1987), and that a "surface area correction" factor of 12.7 should be applied to account for certain expected diferences in carcinogenic potency between species. Application of the surface area correction factor is controversial, however. It can be argued that the pharmacokinetic model leads to risk estimates 111-fold lower by Method 1 (8.8 x 12.7) or 27-fold lower by Method 2 (2.1 x 12.7), than under the applied-dose method. The fact that there is no clear basis for choosing to use surface area correction or not (or for choosing some other method of cross-species extrapolation of risks from internal dose) is a weakness of the current state of the art of quantitative risk assessment.

In vitro data supplied by CEFIC (1986e) suggest that the kinetic parameter for the pathway in the model used by Andersen and Reitz may be too high. A lower value would result in further lowering the estimated human risk. EPA cannot place a great deal of confidence in any quantitative use of these in vitro data in risk estimation, however, because the method for estimating in vivo metabolic kinetic parameters from in vitro data is not well

defined, and such risk estimates do not, as yet, take into account changes in the pharmacokinetic model required by the adjustment of a particular kinetic parameter.

A comparison of risk estimates made directly from the human data provided by the Kodak epidemiology study to risk estimates derived from the results of the pharmacokinetic model used by Andersen and Reitz does not show the animal-based risk estimates to be overestimates, using upper-bound risk estimates from respiratory cancer deaths or using either maximum likelihood estimates or upper-bound estimates from the pancreatic cancer deaths in the Kodak study.

In summary, EPA concludes that the animal evidence of carcinogenicity conforms to the definition for "sufficient" in the EPA Guidelines for Carcinogen Risk Assessment. The epidemiology studies, while showing no evidence of either liver or lung cancer attributable to DCM, are not sufficient to rule out a risk to humans; the data on deaths from pancreatic cancer give some weight to the possibility that DCM may cause cancer in humans at sites other than those found in animal species. Overall, the epidemiologic data conforms to the definition in the Guidelines for "inadequate" insofar as the pancreatic cancer deaths cannot be used to establish a connection between exposure to DCM and human carcinogenicity, yet neither can the possibility of a such a connection be entirely discounted. Thus, DCM meets the Guidelines criteria for Group B2, probable human carcinogen.

The available body of evidence on the carcinogenic mechanism

of action of DCM and on species differences in utilization of the carcinogenic metabolic pathway are not sufficient to support an estimate of zero cancer risk to humans. An evaluation of the weight of evidence does lead to the conclusion, however, that risks should be estimated on the basis of internal dose of the GST metabolite(s). A comparison of the results of the available studies indicates that the GST pathway is the most likely source of the excess tumorigenesis observed in the NTP mouse bioassay.

Additional research on pharmacokinetic model parameters and on the carcinogenic mechanism of action underway by CEFIC are expected to lead to refinement of the risk estimates presented in Chapter 8. It should be noted that data from the experiments may lead to human risk estimates below those estimated from the currently available data. To the degree that the estimates of relative metabolism by the GST pathway change as a result of these data, the extrapolated risks will change. (Method 2, which does not use metabolism to extrapolate across species, would be expected to give virtually the same risk estimates as before, irrespective of interspecies differences in metabolism that may be discovered.)

Using the pharmacokinetic model with its original kinetic parameters to estimate the internal dose of the GST metabolite, then following Method 1, and correcting internal dose for interspecies differences in sensitivity by using the surface area correction factor, leads to a unit risk estimate for continuous inhalation exposure to 1 ug/m^3 of 4.7×10^{-7}.

It would be unwise to read too much importance or significance into changes in the unit risk of a few fold when pharmacokinetic data are employed by either Method 1 or Method 2. The previous chapters have outlined uncertainties in the structure and parameter values of the model formulated by Andersen et al. (1986, 1987). Although it is difficult to define these uncertainties in quantitative terms (such as confidence limits), it is clear that model projections of internal doses could vary, perhaps by up to several fold, without contradicting currently available model validation data. Moreover, there are large uncertainties as to the biological effects of those internal doses that overshadow any error in their estimation. Species differences in responsiveness--and within-species differences in susceptibility of various tissues--are unclear. Perhaps the largest uncertainty lies in the question of the relative carcinogenicity of high and low doses, owing to the lack of knowledge about the mechanism of DCM's carcinogenic action. It is somewhat ironic that the area of risk extrapolation that has the least uncertainty as far as pharmacokinetics is concerned--relative internal doses at high and low exposures--also has the greatest uncertainty in terms of the degree of carcinogenic response that those internal doses can be expected to engender. (Such uncertainty continues to be accommodated by the use of an upper-bound, linearized multistage model for low-dose extrapolation, which recognizes that the true dose-response curve may fall off more rapidly at low doses.)

In view of the uncertainties involved, the changes in DCM's carcinogenic potency that result from different uses of the available pharmacokinetic information are not, in practical terms, very distinct. Discussion of the issues has been worthwhile because of their theoretical importance rather than their practical significance in the present case. For other compounds (or for DCM itself, upon the introduction of new data), the distinction among extrapolation methods may have much greater practical consequences.

Rather than focusing on exactly how much the risk extrapolation has been changed by the use of pharmacokinetic information, it is instructive to examine how little it has been changed. Perhaps the most important result of the foregoing analysis is that, in the case of DCM, pharmacokinetic considerations have not revealed a great error inherent in using applied dose as a surrogate for internal or delivered dose. According to current understanding as expressed in the pharmacokinetic model used by Andersen and Reitz, there is little difference between mice and humans in the proportion of a given applied dose that is metabolized. There are differences in this proportion from high to low doses, but they are not especially large. Having uncovered these pharmacokinetic factors, it is well to incorporate our best understanding of them into the risk extrapolation process, despite remaining questions as to their exact magnitudes. The uncertainty in the resulting potency estimates is reduced (compared to the extrapolation based on

applied dose) because the potential for the influence of pharmacokinetic factors has been markedly circumscribed.

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