

Research and Development



Technical Analysis of New Methods and Data Regarding Dichloromethane Hazard Assessments

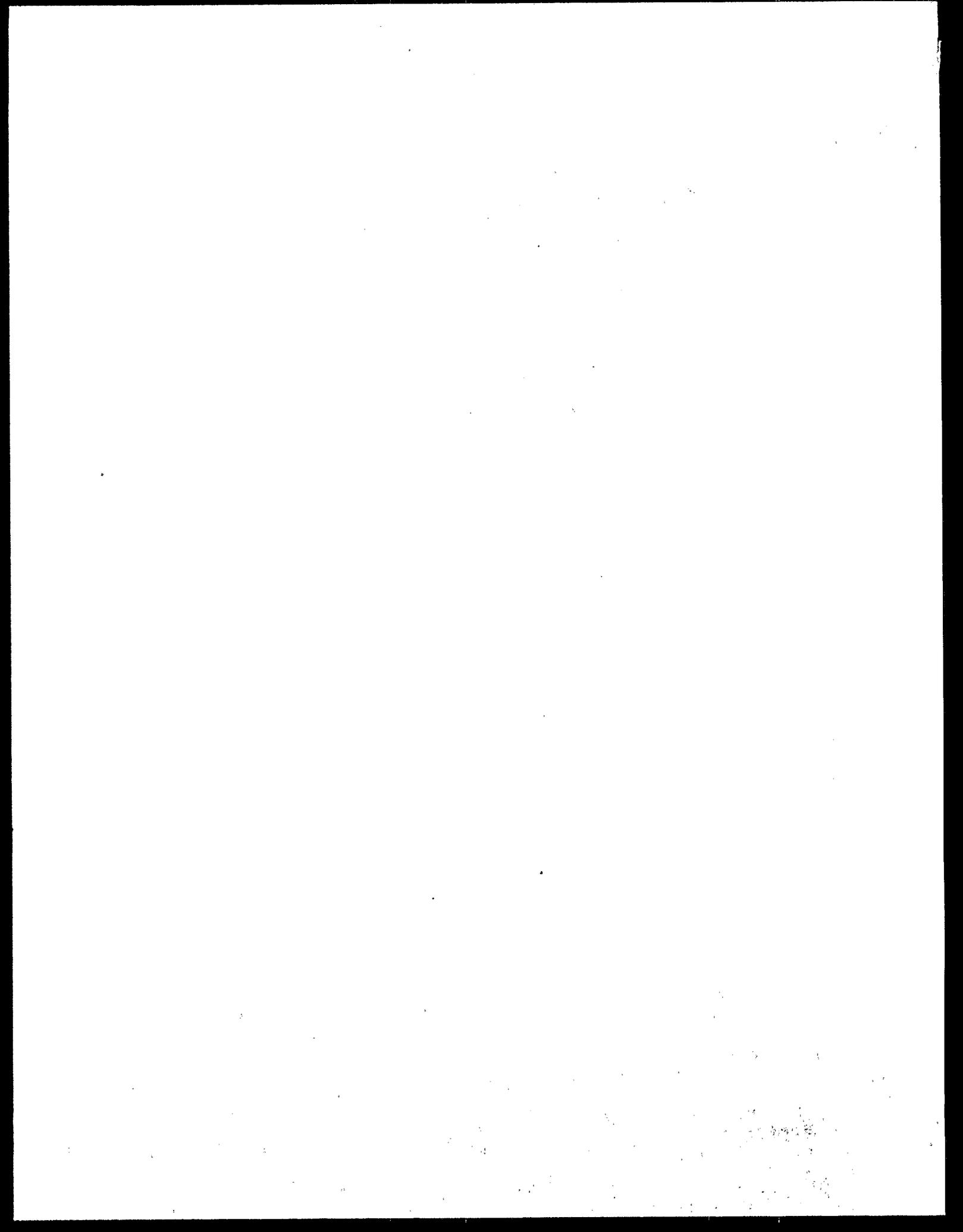
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Review Draft

TECHNICAL ANALYSIS OF NEW METHODS AND DATA
REGARDING DICHLOROMETHANE HAZARD ASSESSMENTS

Prepared by:

The Interagency Hazard/Risk
Assessment Committee of the Integrated
Chlorinated Solvents Project

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PREFACE

This document was prepared by the Health/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project, a committee comprised of representatives from four federal regulatory agencies. This interagency committee was established to evaluate the health effects caused by dichloromethane (DCM) and five other halogenated solvents. As part of its work on halogenated solvent compounds, the HRAC reviewed and evaluated the information recently submitted to EPA and other federal agencies on DCM's potential to cause cancer and other toxic effects.

This document provides an extensive analysis of the new data that have become available since the publication of EPA's Health Assessment Document (HAD) for Dichloromethane (DCM) and Addendum in 1985, and also discusses alternative methodologies for determining risk for DCM. Thus, this document is not intended to replace the 1985 reports, but to provide an evaluation of the recent data and risk assessment methodologies. Its purpose is to provide the agencies with the latest background information on DCM that can be used by each agency in developing its own risk assessment.

ABSTRACT

New information on cytotoxicity, genotoxicity, and epidemiology has raised some questions about the federal regulatory agencies' cancer risk assessments for dichloromethane (DCM, methylene chloride). In addition, physiologically based pharmacokinetic models have been developed, showing that tissue-level delivery of metabolically activated DCM may be disproportionately reduced at low exposure levels. These studies suggest to some that the clear carcinogenic response seen in mice under chronic high exposures does not imply substantive human risk at low doses.

The Health/Risk Assessment Committee (HRAC), comprising representatives of four federal regulatory agencies, was convened to conduct joint analyses of these new data. This document reports on the HRAC's consideration of the data and the questions they raise about human cancer risk from DCM. It serves as a source of up-to-date analyses that may be drawn upon by each agency as it considers modifying its cancer risk assessment.

The HRAC finds that, despite new data, the mechanism of carcinogenic action of DCM remains problematical; there is no basis at present to conclude that carcinogenic response is unique to mice or confined to high exposure levels. Uncertainties in current pharmacokinetic models for DCM are examined, and their application to extrapolating animal-based risks to humans is discussed extensively. Negative epidemiologic studies do not

contradict the human risk estimates extrapolated from
experimental animals.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

This document represents the joint efforts of scientists from several federal regulatory agencies who are members of the Hazard/Risk Assessment Committee of the Integrated Chlorinated Solvents Project.

EPA's Office of Health and Environmental Assessment had overall responsibility for coordination and direction of the document preparation and production effort (Jerry N. Blancato, Project Manager).

AUTHORS

| | |
|--|----------------|
| Steven Bayard Carcinogen Assessment Group Office of Health and Environmental Assessment U.S. Environmental Protection Agency | Chapter 6 |
| David L. Bayliss Carcinogen Assessment Group Office of Health and Environmental Assessment U.S. Environmental Protection Agency | Chapter 6 |
| Jerry N. Blancato Exposure Assessment Group Office of Health and Environmental Assessment U.S. Environmental Protection Agency | Chapters 1 & 2 |
| Miriam Bloom U.S. Consumer Product Safety Commission | Chapter 5 |
| Murray Cohn U.S. Consumer Product Safety Commission | Chapters 4 & 7 |
| William H. Farland Office of Health and Environmental Assessment U.S. Environmental Protection Agency | Chapter 5 |

David Jacobson-Kram
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency

Chapter 5

Lorenz Rhomberg
Carcinogen Assessment Group
Office of Health and Environmental Assessment
(formerly with the Office of Toxic Substances)
U.S. Environmental Protection Agency

Chapter 7

Hugh L. Spitzer
Office of Regulatory Analysis
U.S. Environmental Protection Agency

Chapter 3

CONTRIBUTORS

Robert Brown
Food and Drug Administration

Jane Hopkins
Office of Solid Waste and Emergency Response
(formerly with the Office of Toxic Substances)
U.S. Environmental Protection Agency

Ronald Lorentzen
Food and Drug Administration

REVIEWERS

The following individuals reviewed earlier drafts of this document and provided valuable comments.

Karl P. Baetcke
Office of Toxic Substances
U.S. Environmental Protection Agency

Diane D. Beal
Office of Toxic Substances
U.S. Environmental Protection Agency

Karen Blanchard
Office of Air Quality Planning and Standards
U.S. Environmental Protection Agency

Chao W. Chen
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency

Margaret M.L. Chu
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency

Fred DiCarlo
Office of Toxic Substances
U.S. Environmental Protection Agency

Amanda Edens
Occupational Safety and Health Administration
U.S. Department of Labor

Ernest Falke
Office of Toxic Substances
U.S. Environmental Protection Agency

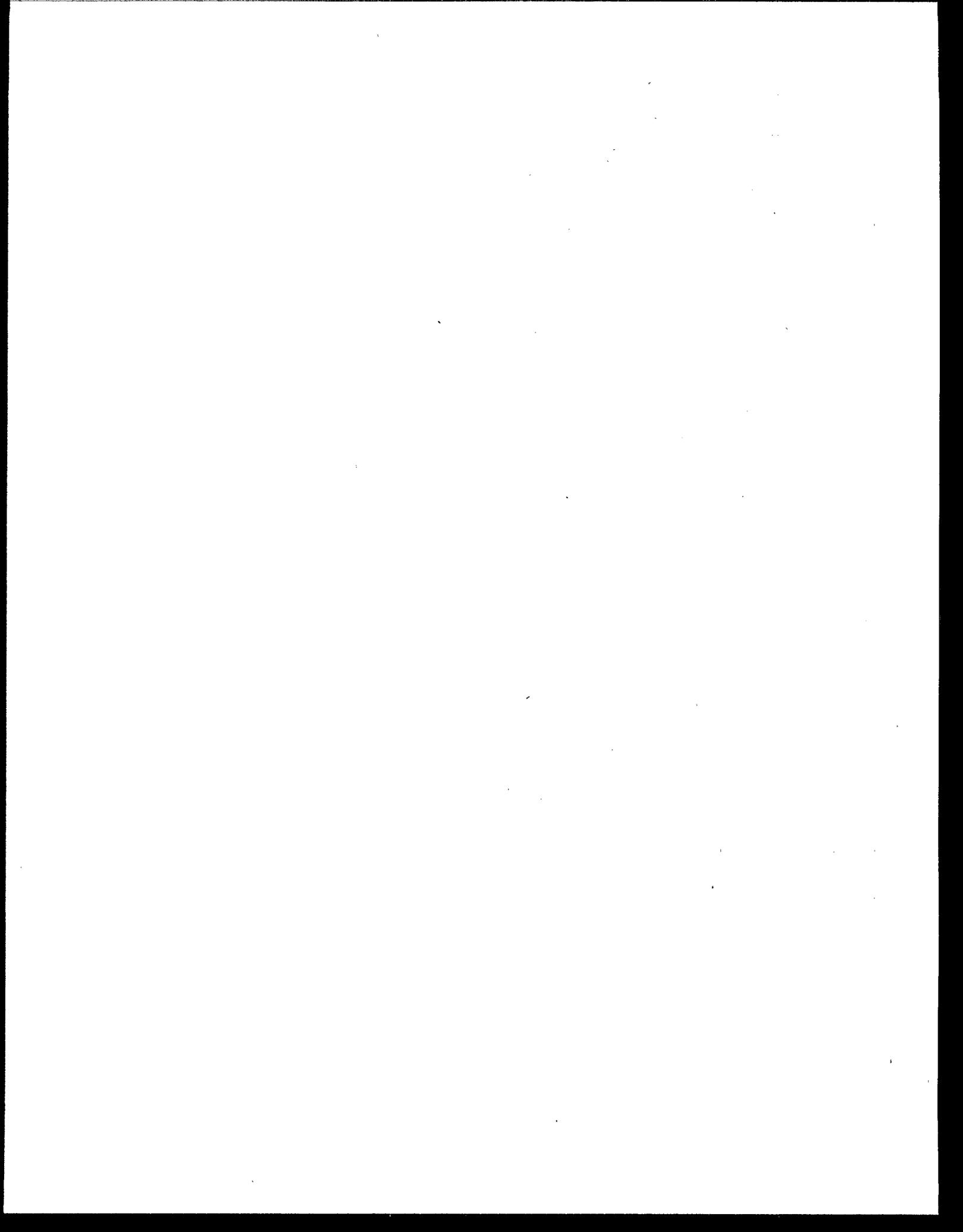
Richard N. Hill
Office of Pesticides and Toxic Substances
U.S. Environmental Protection Agency

Charalingayya B. Hiremath
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency

Carl Mazza
Office of Toxic Substances
U.S. Environmental Protection Agency

Paul White
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency

Harold Zenick
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency



1. INTRODUCTION

This document is the product of the Hazard/Risk Assessment Committee (HRAC) of the Integrated Chlorinated Solvents Project. The following federal regulatory agencies participated in this effort: the U.S. Consumer Product Safety Commission (CPSC), the U.S. Environmental Protection Agency (EPA), the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services, and the Occupational Safety and Health Administration (OSHA) of the U.S. Department of Labor. Scientists from these agencies cooperated in an intense effort to review the numerous technical papers on dichloromethane (DCM, methylene chloride) that had been submitted to the agencies or published since the publication of EPA's Health Assessment Document (HAD) for Dichloromethane and Addendum in 1985. The chapters that follow report on the extensive analyses of the new data and alternative methodologies for determining risk for DCM.

This document does not replace previously published documents, nor is it a risk assessment per se. It is the HRAC's intention that this document be used as background when each agency develops its most up-to-date risk assessment for DCM for its own mandated purpose.

Chapters 2 through 4 review the physiologically based pharmacokinetic models used by Andersen et al. (1986, 1987) and Angelo et al. (1984) to describe and attempt to predict the disposition of DCM and its metabolites in the body. An ultimate

goal of such models could be to quantitatively account for interspecies differences in metabolism and pharmacokinetics. Also, the models can be employed to account for pharmacokinetic nonlinearities that arise when dose-to-dose extrapolations are performed in the risk assessment process.

Although the models are capable of predicting some facets of DCM disposition, several concerns result regarding the structure and parameters of the models. The most crucial uncertainty appears to revolve around the estimates chosen for the key rate constants in the metabolic pathways that transform DCM into other products, including putative carcinogenic species. Other studies, submitted by the European Council of Chemical Manufacturer's Federation (CEFIC, 1986a, e, f), were also extensively reviewed in an attempt to reduce the uncertainty associated with the metabolic parameters.

Chapter 5 reviews several submitted studies (CEFIC 1986b, c, d, g) regarding DCM's potential mechanism of carcinogenicity. Although not in themselves conclusive, the results of these studies could be consistent with considering DCM to be a weak genotoxic agent.

A recent update of the epidemiology of Kodak workers with known exposure to DCM (Hearne et al., 1987) was reviewed in Chapter 6. The quality of the study was assessed and the study results were compared for consistency with risks calculated from animal-based experiments. The HRAC concluded that the risks calculated from animal-based experiments can be considered to be

consistent with the results of the epidemiology studies.

Chapter 7 illustrates two possible applications of how pharmacokinetic information and data may be incorporated into the quantitative risk assessment. One method incorporates interspecies differences in pharmacokinetics while the other only incorporates differences resulting from high- to low-dose extrapolation. The major uncertainties associated with each approach are discussed. For the present, when using the pharmacokinetics for high to low dose only, the estimated upper bound on the risk would be reduced from the applied dose estimates, at a minimum, slightly more than twofold. On the other hand, using pharmacokinetic data and models for interspecies extrapolation would result in a reduction of risk from the applied dose estimate by almost ninefold. The two methods differ in the assumptions that are made and are not equally sensitive to one of the key metabolic rate constants. For example, the ninefold reduction resulting after incorporating pharmacokinetics into the interspecies extrapolation could be substantially altered with alternative estimates for the metabolic rate constant for the conversion of DCM by a glutathione-S-transferase (GST) mediated pathway.

In formulating methodology to incorporate pharmacokinetic information into risk assessments, a number of generic questions are revealed. The relative importance of interspecies differences in pharmacokinetics and pharmacodynamics will have to be discerned to further reduce uncertainty in the risk assessment

process. Pharmacokinetic models may not necessarily settle all the questions regarding equivalency of doses between different species; however, valuable insight regarding the magnitude and consequence of metabolic and pharmacokinetic differences between species can be gained from their use. Exposure-related questions, such as comparing the effect of sporadic high level with low level sustained exposure can be more readily and accurately answered using pharmacokinetic models.

Pharmacokinetic data and pharmacokinetic models do not answer all of the "old" questions that have faced previous risk assessors but hopefully provide new insights into understanding how major uncertainties can be reduced.

2. PHARMACOKINETICS AND PHARMACOKINETIC MODELS

2.1. INTRODUCTION

One of the most challenging issues concerning the HRAC's risk assessment of DCM has been with respect to pharmacokinetics. It is very important to ascertain whether or not the estimate of risk for this compound is appreciably altered after considering pharmacokinetic data. Basically two physiologically based pharmacokinetic (PBPK) models have been formulated to predict the disposition of DCM and its metabolites in the body. The first model is based on an earlier model formulated to describe the disposition of styrene (Ramsey and Andersen, 1984). This model has been modified by Andersen et al. (1986, 1987) and Reitz et al. (1986) to account for DCM exposure. Earlier drafts of these studies were supplied to the various federal regulatory agencies for review. It is postulated by Andersen et al. (1986, 1987) and Reitz et al. (1986) that if the EPA considers the results of this model, the risk number would be greatly reduced from what is presently in the HAD for DCM.

A second PBPK model formulated by Angelo et al. (1984) has also been used to describe the disposition of DCM and its metabolites in the body. A series of accompanying papers have been published (Angelo and Pritchard, 1984; Angelo et al., 1986a, b) that provide data on several important pharmacokinetic considerations. After reviewing both the Andersen and Angelo papers, it becomes quite obvious that the two models have

significantly different structures. This chapter will discuss the use of pharmacokinetics in risk assessment and will examine both models and elucidate differences between the two. It will also assess some of the assumptions that have gone into the formulation of the models.

2.2. PHARMACOKINETIC MODELS AND METABOLISM

2.2.1. Model Used by Andersen and Reitz

This PBPK model for DCM is based on a similar model published by Ramsey and Andersen (1984) to describe the disposition of styrene. The model has been modified for the case of DCM (Andersen et al., 1986, 1987) to account for metabolism by two pathways. Both metabolic pathways are assumed to occur in the liver as well as in the lung. One path is mediated by the P-450 system and is considered to exhibit saturation kinetics at the given exposures. The second pathway is mediated by the glutathione-S-transferase (GST) system. This pathway is considered to be first order at the given exposure conditions. Most importantly, it is assumed by Andersen et al. (1986, 1987) and Reitz et al. (1986) that this pathway is the only source of carcinogenicity. Further, it is postulated that the activity of this pathway in humans is less than that of mice and might only become significant when the P-450 pathway has been saturated, a process occurring only at concentrations above those expected in conditions of human exposure. Reports presented by CEFIC (1986e) further imply that the activity of this pathway in humans is at least two orders of magnitude lower than in mice, and in fact,

may be totally nonexistent. Thus, it is inferred that human risk is practically zero.

For the inhalation case the model assumes that the inhaled air in the lung and pulmonary blood quickly achieves and maintains steady-state conditions throughout the course of exposure. None of the other organs are assumed to be in steady state. This steady-state assumption is reasonable for this type of compound under the conditions of exposure outlined by Andersen et al. (1986, 1987). The model is structured so that the lung is divided into two subcompartments: a gas exchange compartment and a metabolism compartment. The lung was modeled with this subcompartmentalization because the DCM rapidly equilibrates between air and lung blood. Thus, it was assumed that this equilibration was completed before the DCM entered the lung tissue (Andersen et al., 1987). Given, the possibly high metabolic activity of some of the lining cells (CEFIC, 1986a), this may not be a totally accurate picture of the lung. However, it is probable that error, if introduced, would be small.

Some key physical parameters that go into the model are partition coefficients. These partition coefficients actually represent the ratio of distribution of DCM between a tissue compartment and the blood at conditions where the blood and the tissue are at equilibrium or between blood and air. Although they may be determined in a variety of ways, often partition coefficients are determined in an experimental situation with dosing performed so that blood concentrations are at steady

state, such as may be achieved with a constant infusion. Alternately, they are determined mathematically from data obtained at various times after exposure. Usually these data should be the result of exposure at more than one dose. The method used by Andersen et al. (1986, 1987) for DCM, however, is significantly different. Tissues are homogenized and then partition coefficients are determined with a vial equilibration technique. This essentially means that air/tissue partition coefficients are determined for various tissues including the blood. A ratio of the air/blood to air/tissue coefficient is then the tissue blood/partition coefficient. Considerable concern arises over whether or not this results in an accurate determination of this parameter.

Essentially, distribution of a compound among different compartments is governed by its reversible binding with proteins and/or other constituents and permeability of the various membranes across which it may pass (Terasaki et al., 1984). The homogenization of tissues clearly alters their normal architecture and thus leads to several experimental problems that could effect the accuracy of the resultant partition coefficients. For example, it is not clear how, after the homogenization process, artifacts such as disruption of normal membranes, degeneration of normal binding components, and altered metabolism should be taken into account.

Angelo et al. (1984) used a different method for determining partition coefficients which will be discussed more fully in

subsequent sections. The model used by Angelo et al. uses coefficients for two subcompartments in each organ, a vascular compartment and an extravascular compartment. The extravascular compartment is thought to be perhaps lipid containing. Comparisons of the coefficients reported by Andersen et al. (1986, 1987) paper with those reported for the vascular compartment in the Angelo et al. (1984) paper reveal a close correlation. Thus, in this case it is probable that the partition coefficients used by Andersen et al. (1986, 1987) are within acceptable ranges for describing partitioning across the vascular membrane. It is possible that some inaccuracy could result if an organ has a significant lipid fraction and partitioning occurs from the vascular region into the lipid region. If tissue and blood values for DCM were determined and reported over time, the distribution values could be estimated by other means (King et al., 1983), and thus a more direct comparison of in vivo and in vitro values could be made. In addition, in a memo sent to the U.S. EPA dated March 9, 1987 (TSCA docket no. OPTS-62045), Harris reports that work done at the Central Toxicology Laboratory of the Imperial Chemical Industries-United Kingdom (ICI-UK) shows that rats and mice have the same air/blood partition coefficient, in contrast with the differences reported by Andersen et al. (1986, 1987). The actual quantitative impact that this discrepancy could have on the model has not yet been determined, but it does raise some questions about the methods used by Andersen et al. (1986, 1987). In fact,

in earlier discussions ICI-UK scientists stated that in order for the model to coincide with data generated in their laboratories, the fat/blood partition coefficient had to be raised almost an order of magnitude. This might make the value for this parameter in excess of expected and other reported values. Also, a combination of errors in the metabolic rate constants and the partition coefficients may cause the model used by Andersen et al. (1986, 1987) to be in error. While few definitive conclusions can be drawn at this time, the sensitivity of the model to these parameters serves to demonstrate that PBPK models depend on a complex set of parameters. Because of the interactions of those parameters within the model, estimating error for any one of the parameters can become a very difficult task.

A short discussion regarding the breathing rate parameters that have been used by Andersen et al. (1986, 1987) and Reitz et al. (1986) is warranted. 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 assumption, while that for humans is markedly lower. The Andersen value for humans is for a person at rest, but the EPA, FDA, and CPSC use a value considered typical of average activity (almost twice as high as Andersen's value) or occupational activity (nearly three times higher than Andersen's value). The values chosen by Andersen et al. (1986, 1987) and Reitz et al. (1986) are not necessarily incorrect but apply to the specific exposure conditions that

those experimenters observed for a given set of experiments. To compare any estimate of body burden or risk estimation with that typically calculated by the federal regulatory agencies, the breathing parameters should be the same for both methods.

Andersen et al. (1986, 1987) compared the federal regulatory agencies' prediction of risk with predictions based on the PBPK model using the original breathing rates; i.e., risks based on the higher human breathing rate without pharmacokinetics have been compared to risks based on the lower breathing rate with pharmacokinetics. This issue is discussed further in Chapter 7 which contains an evaluation of the effects of the PBPK model on the risk assessment.

Another key set of parameters is the metabolic constants, which are discussed in more detail in subsequent chapters. Briefly, Andersen et al. (1986, 1987) and Reitz et al. (1986) applied their model to data on the disappearance of DCM from a closed inhalation chamber, as the compound is taken up and metabolized by mice, rats, and hamsters. They developed values on the rates of metabolism by each pathway for the model, not from direct experimentation but by mathematical optimization, to provide the best fit of the model to the data on the disappearance of DCM from the chamber. Because human data were judged to be inappropriate for the optimization routine, the value for the human GST metabolism constant (k_F) was determined by scaling based on body weight to a power. Andersen et al. (1986, 1987) observed that clearance (mL/hr) for the GST pathway

in the liver appeared to have an allometric relationship in the three rodent species, as demonstrated by the fact that intrinsic clearance (rate constant times the liver volume divided by body weight raised to the 0.7 power) was nearly equal in all three species. The highest experimentally observed intrinsic clearance (60 mL/hr/kg) was then used to estimate the clearance in 70-kg humans. From this clearance, the human k_F was determined. As is discussed in Chapters 3 and 4, the HRAC has some reservations with the method and the results of such an extrapolation. The potential for error in this method is illustrated by the fact that the findings of CEFIC (1986e) contradict the extrapolation prediction of substantial GST metabolism in human liver.

Although the HRAC feels that the sensitivity of the method used by CEFIC (1986e) is very limited, results of those in vitro experiments indicate very little GST activity towards DCM in human liver tissues. However, it is also well to note that no agreed-upon methods exist to extrapolate metabolic constants from in vitro to in vivo either.

As far as the model is concerned, the metabolic parameters are very important. The premise with this model is that the risk is associated with the formation of metabolites in the liver and the lung. Thus, the actual desired and important output of the model is the metabolite production.

It appears from some investigations (Chapter 4) that the varying of the metabolic parameters results in a different prediction of metabolite production while still fitting the data

regarding the disappearance of DCM from the inhalation chamber. The result is that an inaccurate metabolic parameter in the model would not be noticed if model output was compared simply to chamber data. Thus, it is very important to have actual metabolite production data to "test" the model. This is particularly important if metabolite production is key to risk estimation. Although the gathering of preliminary data by Reitz et al. (1986) is a first step in this process, more such data will be needed to properly determine the level of certainty of this model.

Additional key metabolic parameters in the model are those that specify the relative activities of the two pathways between metabolically active organs, in this case the liver and lung. Andersen et al. (1986, 1987) partitioned 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 and 7-ethoxycoumarin for MFO activity). On the basis of the Lorenz data, Andersen et al. (1986, 1987) 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. Concerning GST "surrogates," it is not clear whether or not isoenzymes exist, and if so, whether they would all show the same level of activity. (Specific reviews of this procedure are discussed in Chapter 3.) The model would be very sensitive to

errors in the partitioning of metabolism. In particular, an error in the partitioning of GST activity between liver and lung would result in essentially a proportional error in the model's prediction of metabolite, and thus of the estimated risk.

CEFIC (1986f) have gathered data in vivo in both rats and mice in an attempt to validate existing PBPK models of DCM disposition. Briefly those findings indicate that the disposition in mice is different than that in rats. These studies do substantiate the fact that mice convert DCM more efficiently than do rats. In addition, both species show a two-phase elimination profile after exposure ceases. The slower second phase, much more pronounced in rats, is probably due to release of DCM which had sequestered in lipid-rich compartments of the tissues during exposure. One probable explanation for the longer second phase elimination in rats is that, due to lower metabolic rates, rats tend to sequester the parent compound in lipid-rich compartments during exposure and slowly release it after the exposure period has terminated. These differences between rats and mice are observed at levels above which mechanisms for production of carbon monoxide formation from DCM are saturated.

These data further indicate that after saturation of the carbon monoxide pathway in rats, there is a nearly linear increase in the area under the curves (AUC) for DCM in the blood with increasing exposure concentration. On the other hand, in the mouse, the AUC for DCM in the blood does not show a linear

relationship with exposure concentration but rather an increase in a sublinear fashion. Thus, it appears that the ratio of the V_{\max} for the P-450 pathway and the k_F (first-order rate constant) for the GST-mediated pathway is different in the mouse as compared to the rat. Therefore, the finding of linearity of the blood concentration of the parent compound with increasing exposure concentration in the rat, but not in the mouse, need not necessarily lead to the conclusion that no GST activity occurs in the rat. Rather, it could simply mean that the ratio of V_{\max} to k_F is greater in the rat than in the mouse.

Careful examination of the profiles of DCM in the blood of both rats and mice reveals additional interesting findings. It appears that at the lowest exposure concentration (500 ppm) rats are more efficient at removing DCM from the blood than are mice. Yet, at this 500-ppm exposure both species show the same level of saturation of hemoglobin with carbon monoxide. Thus, it might be deduced that rats, at low exposure conditions, have some mechanism in addition to P-450 metabolism to remove DCM from the blood. There are two possible explanations for this more efficient blood removal of the parent compound at low exposure concentrations by the rat. First, the rat is able to sequester more of the parent compound in fat-containing regions of the body. Angelo et al. (1984) found that DCM elimination could only be explained (after intravenous dosing in a vehicle containing polyethylene glycol and water) if some sequestering into lipid-rich compartments of the organs were considered. A second

possibility is that rats show some other metabolic process at very low exposure concentrations. However, it is clear from examining carbon dioxide elimination patterns that carbon dioxide-producing pathways, such as the GST pathway, although present, are not as active even at low doses in rats.

An apparent explanation for at least part of this more efficient elimination of DCM from the blood by rats is that due to a larger fat compartment, they are able to sequester the compound more efficiently at these lower concentrations than are mice. Also, the "filling" of the fat compartment can be thought of as a saturation of the another process of eliminating DCM from the blood. It appears that both the fat compartment and the P-450 metabolism mechanism are saturated by 1000 ppm. In conclusion, these data strongly suggest that the metabolic capabilities of mice are different than those of rats regarding DCM. Mice are able to continue metabolizing the compound even after the oxidative carbon monoxide-producing pathway has been saturated, as opposed to rats which appear to have a lower level of activity of the GST pathway at the tested exposure conditions.

CEFIC (1986f) has not reported on any comparison of their in vivo data with predictions of the model of Reitz et al. (1986). However, in discussions with the HRAC they have stated that the fat/blood partition coefficient had to be increased by an order of magnitude in order for the model to fit. This increase would be beyond any expected range for the value for this parameter. Thus, it is apparent, according to those workers, that some other

parameter in the model must be in error, or that the model's structure is in error. Green and coworkers are under the assumption that the metabolic parameters selected by Reitz et al. (1986) are in error, and have embarked on a series of experiments that may greatly aid in establishing more accurate values for those parameters. In general, the HRAC agrees that any error in the model is likely to be with the metabolic parameters; however, a question remains regarding partition coefficients (section 2.2.1.) and the model's structure. This question arises when comparing the model used by Andersen et al. (1986, 1987) with one developed by Angelo et al. (1984) for DCM after intravenous and per os exposure (discussed more fully in the next section).

2.2.2. Model Used by Angelo et al.

The PBPK model used by Angelo et al. (1984) is structured somewhat differently from the model used by Anderson et al. (1986, 1987) in that it was formulated to describe the disposition of DCM after an intravenous dose. The model also takes into account metabolism to carbon dioxide and carbon monoxide by two pathways. However, this model considers most of the body organs to have two subcompartments. One, termed the vascular region, is blood flow limited, and the other, termed the extravascular region, is membrane diffusion limited. This model is somewhat more complex than the model used by Andersen et al. (1986, 1987), and more parameters are required as input. The structure of the model was found to best describe the data gathered after an intravenous exposure.

In the model used by Angelo et al., the partition coefficients are determined by a much different process than those in the model used by Andersen et al. (1986, 1987), that is, they are determined mathematically by optimizing from several time points at two doses. This method is used for a variety of reasons. The most important reason is that for each organ the partition coefficient for the lipoid region would be very difficult to determine experimentally. This method is similar to that used by King et al. (1983) and it appears to be a reasonable way to determine the partition coefficients. It would be interesting to have had an experiment in which the whole organ partition coefficients were determined from a steady-state infusion technique. The organ partition coefficients could then have been compared to the vascular region coefficients in the model used by Angelo et al. as well as those derived from the equilibration technique in the model used by Andersen et al. (1986, 1987).

Pulmonary clearance rates, metabolism constants, and permeability-area products are determined from in vivo data. Pulmonary clearance of DCM is calculated by dividing the total amount excreted from the lung following a 60-minute time period after dosing by the integral of arterial blood concentration (Angelo et al., 1984). The amount of DCM that is excreted is determined experimentally. The metabolic clearance is determined in a similar fashion using the total amount of each metabolite produced (carbon dioxide and carbon monoxide) divided by the

integral of the venous concentration of DCM in the venous blood. The apparent in vivo metabolism constants (K_M and V_{max}) are then mathematically derived from these data. The permeability-area products are determined from experimental data. The data needed to determine the metabolic constants are the venous blood concentration of DCM, the total organ concentration of DCM, and the integral of the venous concentration of DCM. These are considered initial estimates for all of the parameters. Then, given the data from two doses, an optimization routine results in a "final fit" for all.

Given intravenously, DCM appears to sequester in certain tissues while simultaneously disappearing from the blood. In subsequent work (Angelo et al., 1986a, b) this phenomenon was investigated further. After reviewing these papers, it does indeed appear that DCM remains in some of the tissues for quite some time after its disappearance in the blood. Angelo et al. (1986a, b) concluded that this is due, for some reason, to the vehicle of administration. They reported that, when dosing by the oral route with an aqueous vehicle, the disposition profile is not the same as when dosing by an intravenous route with a polyethylene glycol vehicle.

A strong point of the model used by Angelo et al. is its favorable comparison to the data. The model was particularly successful in predicting the disposition of DCM, carbon dioxide, and carbon monoxide for repeated oral dosing. It also predicted some components well after an intravenous dose. The predicted

values for DCM elimination from the lung were 10% to 15% higher than the actual data. The prediction for carbon dioxide and carbon monoxide elimination were excessive at short times but closer at predicting the ultimate amounts of the formation of these substances.

Predictions using this model indicate that DCM, when given repeatedly for 14 days to Fisher 344 rats by daily oral gavage in water at a dose level of 200 mg/kg, did not sequester in the liver from day to day (Angelo and Pritchard, 1984). That is, by the time of the next daily gavage the previous day's material had been eliminated from the liver. The prediction, therefore, appeared to agree with the data. Further, the model showed that a corn oil vehicle would greatly increase the time required for elimination to occur in the liver compared to an aqueous vehicle. The model's prediction showed the corn oil effect to be less pronounced for venous blood. Thus, blood profiles are not necessarily accurate reflections of tissue disposition profiles. This point needs to be considered when comparing model output to data. Agreement of model output with blood data is not always indicative of agreement with tissue data.

Angelo et al. (1986a) found, upon comparing profiles after intravenous dosing in water with oral dosing in water, that the elimination phase of DCM in the blood is similar for both routes of administration. However, the tissue concentrations were found to significantly differ between intravenous and oral routes.

Intravenous administration resulted in elevated tissue levels over time in the lung and kidney, but not in the carcass. Also of note is that following an intravenous administration, blood profiles do not resemble tissue profiles as they do following oral administration. Thus, it could be misleading to infer tissue disposition merely from blood profiles in the case of intravenous administration. It is not clear at this time whether or not this lack of correlation between blood and tissue occurs after inhalation exposure. This again illustrates some problems when measuring the accuracy of a model against data; that is, a model predicting blood data may not be accurately predicting tissue data.

The effect of route on metabolic profiles is also of obvious consequence when comparing intravenous versus oral administration. Because the liver has greater metabolic activity than the lung, a greater fraction of DCM is metabolized upon "first pass" after oral administration. Thus, less unchanged DCM is available for pulmonary excretion when compared to intravenous administration. In this latter case the lung is the "first pass organ," and thus a greater fraction of DCM can be eliminated unchanged.

The model used by Angelo et al. (1984) was modified to account for inhalation exposure (Angelo et al., 1986c), and the authors report that the model described the distribution of DCM in the blood of rats during a 6-hour inhalation exposure using the data of McKenna et al. (1982). However, the results of that

simulation are not provided (Angelo et al., 1986c). Assuming the simulations accurately describe the data, one must question whether a model, such as the one used by Andersen et al. (1986, 1987), which does not account for sequestering into lipid-rich compartments of some organs, is accurate.

The model was also used to determine "equivalency" between oral and inhalation doses. The correlation varies depending on the target one is observing. The nature of the relationship between routes depends on which tissue, end product, parent, or metabolite is being examined.

The model was able to predict the amount of DCM retained during steady-state inhalation conditions. The predictions were that less than 15% of DCM would be retained in the body at exposure concentrations between 50 and 1500 ppm. Whether or not this indicates error in any previous extrapolation based on applied dose (which assumes 100% absorption) would depend upon whether or not the fraction absorbed is different in experimental animals versus humans and whether or not the absorbed fraction is different at the exposure concentrations of the bioassay from low exposure concentrations. When properly applied, PBPK models are able to account for such differences.

To summarize, three important points can be gained from examining the data and model developed by Angelo et al. (1984; 1986a, b, c) and Angelo and Pritchard (1984). First, tissue concentrations of DCM are not accurately reflected by blood concentrations; thus, to determine how well a model predicts

tissue disposition, tissue data are necessary. Second, it is possible that the disposition of DCM after an intravenous dosing regimen is most likely different from the disposition after an oral dosing. The implication that this difference may have an effect on inhalation dosing is not clear. Third, during steady-state inhalation exposure, much less than 100% absorption would be predicted. PBPK models are able to account for this factor and can account for changes in this factor with different exposure concentrations.

2.2.3. Summary

The model being proposed by Andersen et al. (1986, 1987) and Reitz et al. (1986) needs to be further tested against concentrations of DCM in the tissues. More data regarding the production of carbon monoxide and carbon dioxide are required to further substantiate the model's ability to accurately predict metabolism. Also, these additional data regarding tissue disposition will elucidate whether or not the complexity of the model used by Angelo et al. is necessary.

Further, according to discussions with the ICI-UK scientists, it appears that some reevaluation of certain parameters is necessary. Those investigators feel that the metabolism parameters are in need of readjustment in order for the model to be a valid predictor of tissue concentrations of DCM and its metabolites. The HRAC agrees with this opinion but also feels that there is some possibility that the model may have to be structurally altered to account for sequestering of the parent

compound into lipid-containing compartments. This could be significant when using the model for humans who may have repeated exposure over a long period of their lifetimes.

In addition, several key questions remain, but ICI-UK scientists are conducting studies that may greatly reduce the uncertainties associated with these. First, there remains some uncertainty as to whether or not the GST pathway is the sole path to carcinogenicity. As previously discussed, the evidence to support this assumption is quite strong, but one cannot be 100% certain. Without such assurance some level of uncertainty will always be associated with risk assessments for DCM. When some of the uncertainties with other questions are reduced, it may be possible to estimate the level of certainty with this first assumption which is truly related to the exact mechanism of action. The remaining questions address the activity of the GST pathway. It is obvious at this time that there are species differences in the activity of metabolism by this path. Due to the lack of sufficient sensitivity of the recently completed analyses by ICI-UK, it is not clear whether or not the pathway is totally nonexistent (regarding DCM in rats, hamsters, and humans). If it is proven to be nonexistent and one assumes that this path is the major or only route to carcinogenicity, then the risk estimate to humans could be substantially lowered. It is hoped that ongoing studies at ICI-UK will add a great deal of knowledge in this area. Presently ICI-UK scientists are planning and/or conducting studies using ^{36}Cl -DCM to determine, in vitro,

the levels of GST activity towards DCM in liver, and possibly lung, of various species, including humans. Properly performed, these analyses could answer the question as to whether or not this pathway is active in species, such as the hamster, which have not shown the same tumor response.

Questions regarding the salivary and mammary gland adenomas and possible human pancreas tumors will remain, however, unless the same or comparable pertinent metabolic studies are performed on those tissues as well. The model used by Andersen et al. (1986, 1987) does not, at present, account for "toxic" metabolite production in any tissues except the lung and liver. If other tissues are deemed important in the scheme of potential carcinogenicity to humans, then appropriate parameters would have to be determined and the model modified to describe and predict metabolite disposition in those tissues as well.

The epidemiologic evidence that pancreatic tumors develop after exposure to DCM is equivocal at present. Yet, some studies (Black and Howerton, 1984; Mukhtar et al., 1981) suggest significant GST activity in animal and human pancreatic tissue.

Also, at this time the significance of the rat salivary gland tumors is not considered remarkable, and reports have been published (Russo et al., 1982) which indicate that benign adenomas of the mammary gland are histogenically different from malignant adenocarcinomas and are not believed to have a high malignant potential. A note of caution should be raised here as well. Tumors that are shown to exhibit altered genomes or other

chromosomal aberrations within their cells should be considered to be more significant than those that do not exhibit such changes. These changes, regardless of the tumor type, should be considered relevant when assessing the cancer risk for humans. That is, a substance that may cause a mutagenic change in one mammalian cell should be considered potentially capable of causing such a change in another mammalian cell, including human cells. There are numerous measures to determine whether or not DCM or an active metabolite is mutagenic in specific tissues. Studies to determine the rate of unscheduled DNA synthesis and the amount of covalent binding to DNA have not been performed in mammary cells; thus, it would be impossible to state with absolute certainty that DCM or its metabolites do not have mutagenic potential. However, the mammary tumors in the NTP bioassays were fibroadenomas, and these do not show characteristics indicative of malignant potential. Thus, the significance of these tumors, although uncertain, is probably not great. In fact, Ackerman and Rosai (1974) stated ". . . that the malignant transformation of a fibroadenoma is exceptional and for practical purposes can be disregarded in the management of this lesion. However we have seen a few cases in which part of the epithelial component of a fibroadenoma had the microscopic appearance of carcinoma. The prognosis of tumors limited to the fibroadenomas was excellent."

Additional studies being planned by ICI-UK scientists using the stable isotope effect will provide information necessary to

answer the question regarding the source of carbon dioxide formed after exposure to DCM. CEFIC (1986e, f) and Reitz et al. (1986) assumed that the GST pathway in mice operates only at concentrations that are high enough to saturate the P-450 pathway, and CEFIC (1986e) further assumed that the GST pathway has very little activity in humans. The question then arises as to where the carbon dioxide observed in mice at low doses and rats and humans arise from? CEFIC (1986f) and Reitz et al. (1986) assumed that some carbon dioxide results from metabolism from the P-450 system; thus, the carbon dioxide observed is not inconsistent with the lack of GST activity in rats and humans and lack of GST activity in mice at low doses. Previously it had been assumed that carbon dioxide could only arise from the GST pathway. If correct, this assumption would imply that some GST activity must be occurring in mice at low doses as well as in rats and humans. Determining which of these assumptions is correct could obviously have significant implications on the risk estimate. The planned studies using deuterium replacement for hydrogen, and thus taking advantage of the stronger bond energy that results from substitution, will help to discern the source of the carbon dioxide. Carbon dioxide resulting from P-450 metabolism would be subject to the isotope effect, and such a pathway would exhibit reduced carbon dioxide generation rates. On the other hand, carbon dioxide arising from the GST pathway, due to no carbon deuterium bonds being broken, is not affected in this case, and its production should not be altered by the

isotope effect. Thus, in in vivo studies with deuterated DCM, reduction of carbon dioxide production would be indicative of the amount of carbon dioxide produced by the P-450 pathway. If no reduction of carbon dioxide production is observed, then it can be concluded that the GST pathway is the sole source of carbon dioxide. This would imply that the GST pathway is active at low exposure concentrations in mice and rats. If this is the case, then the structure of the model, its parameters, and its underlying hypothesis must be reevaluated.

To summarize, it is hoped that these studies will serve to remove some doubt as to whether or not the existing evidence can be interpreted to mean that the predominant pathway at low doses is the MFO pathway and at high doses is the GST pathway.

In conclusion, the model used by Andersen et al. (1986, 1987), despite some questions regarding structures and parameter, appears to be a good approach for high- to low-dose extrapolation within a species. The questions and possible errors would not be judged to have a great impact (Chapter 7). However, these errors would have a much greater impact if the model was used for species-to-species extrapolation.

3. METABOLISM OF DICHLOROMETHANE

In a 1985 analysis of the relevant literature, EPA staff scientists concluded that the results of both in vitro and in vivo studies indicated that DCM is metabolized via two pathways (U.S. EPA, 1985a, b). One pathway yields carbon monoxide (CO) as an end product, and the other pathway yields carbon dioxide (CO₂) as an end product. Each pathway involves formation of a metabolically active intermediate that is theoretically capable of irreversibly binding to cellular macromolecules (Ahmed et al., 1980). In vivo data suggest that when rats or mice are exposed to high concentrations of DCM (50 mg/kg or 500 ppm or more), they exhale more CO₂ than CO (Yesair et al., 1977; McKenna et al., 1982). At exposure to low concentrations of DCM (1 to 10 mg/kg or 50 ppm) both pathways are utilized about equally (Yesair et al., 1977; McKenna et al., 1982). These observations are consistent with the data showing that the oxidative pathway (yielding CO) is saturated at relatively low exposures (< 500 ppm) while the pathway yielding CO₂ appears to be first order even at exposures of 1500 ppm.

Recent reports (Gargas et al., 1986; CEFIC, 1986e, f; Reitz et al., 1986) have suggested that: (1) at low doses DCM is almost exclusively metabolized by the oxidative pathway; (2) the oxidative pathway is capable of yielding significant amounts of CO₂ in addition to CO; (3) there is a significant difference in the enzymatic activity of the two pathways from species to

species; and (4) there is a significant difference in toxicity between the reactive intermediates generated by the two pathways. The purpose of this chapter is to review the data used to support these conclusions and to alter the EPA HAD for DCM if needed.

3.1. IN VIVO METABOLISM

The conclusions that DCM is almost exclusively metabolized by the oxidative pathway at low exposure levels and that the oxidative pathway is capable of yielding significant amounts of CO₂ are based on a series of in vivo experiments in rats and mice (Gargas et al., 1986; Reitz et al., 1986).

Gargas et al. (1986) studied the in vivo metabolism of a series of dihalomethanes in rats. These investigators monitored blood bromide during inhalation of dibromomethane (DBM) and bromochloromethane, and blood levels of carboxyhemoglobin (HbCO) during inhalation of DBM, bromochloromethane, and DCM. Gargas et al. (1986) compared control values to those obtained from animals pretreated with pyrazole (inhibits microsomal P-450 oxidation) or 2,3-epoxypropanol (2,3-EP) (which depletes glutathione) in order to assess the relative contribution of each pathway to the total metabolism of dihalomethanes. Animals pretreated with pyrazole had a very significant decrease in blood HbCO during exposure to DBM, DCM, and bromochloromethane (Figures 1, 2, and 3). Animals pretreated with 2,3-EP had a significant increase in blood HbCO during exposure to DCM and bromochloromethane but not DBM. Pyrazole pretreatment only slightly reduced the blood bromide

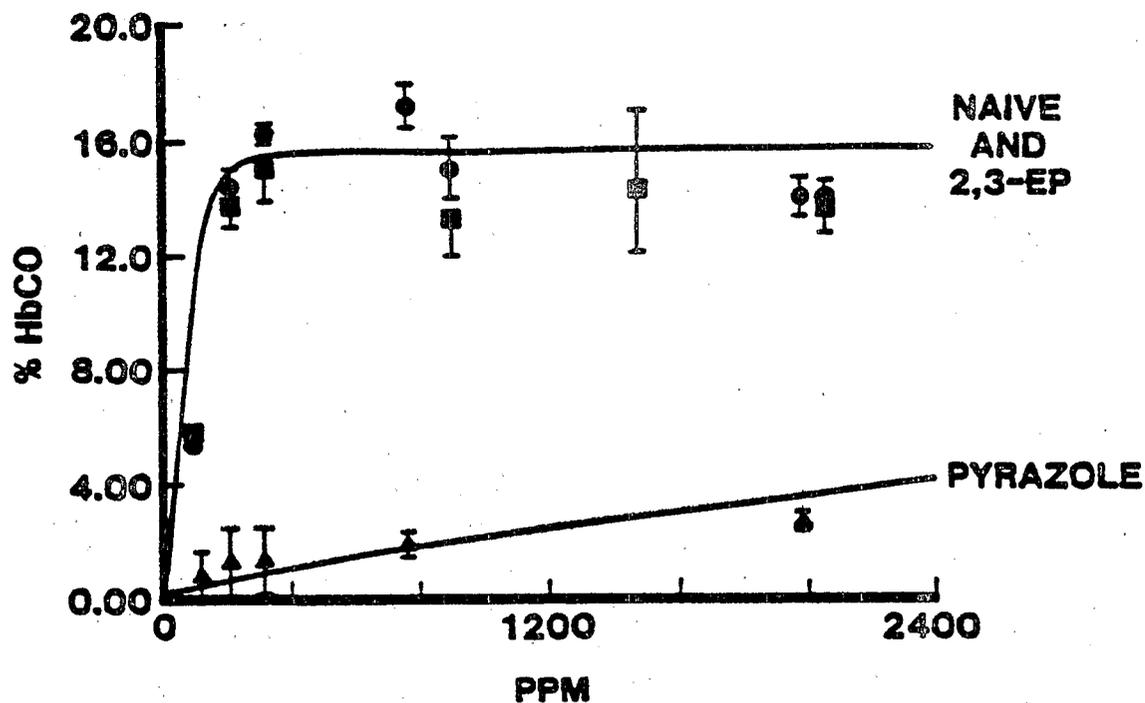


Figure 1. The end of exposure HbCO levels in naive, 2,3-EP, and pyrazole pretreated rats following 4-hour exposures to DBM. Three to six animals were used for each exposure and the smooth curves were predicted by the computer model using the kinetic constants for DBM listed in Table 2. The stoichiometric yield of CO from the oxidative pathway was 100% for both naive and 2,3-EP pretreated rats.

SOURCE: Gargas et al., 1986.

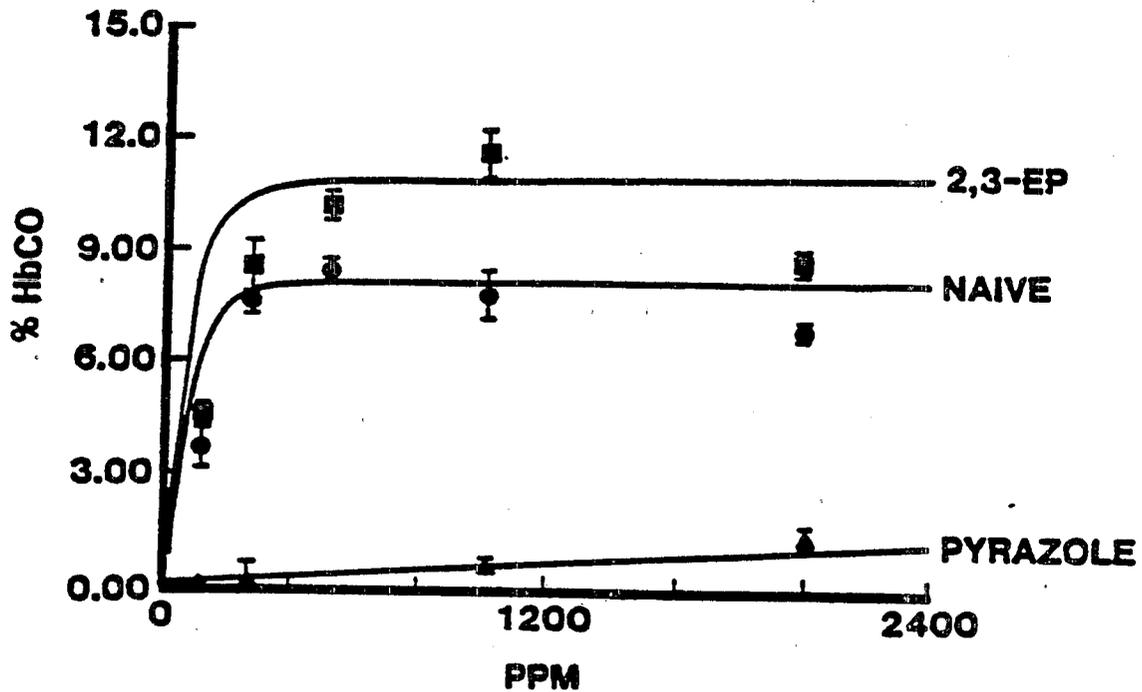


Figure 2. The end of exposure HbCO levels in naive, 2,3-EP, and pyrazole pretreated animals following 4-hour exposures to DCM. Three to six animals were used for each exposure and the smooth curves were predicted by the computer model using the kinetic constants for DCM listed in Table 2. The stoichiometric yield of CO from the oxidative pathway was 100% for 2,3-EP pretreated rats and was lowered to 70% for naive rats.

SOURCE: Gargas et al., 1986.

CH₂BrCl

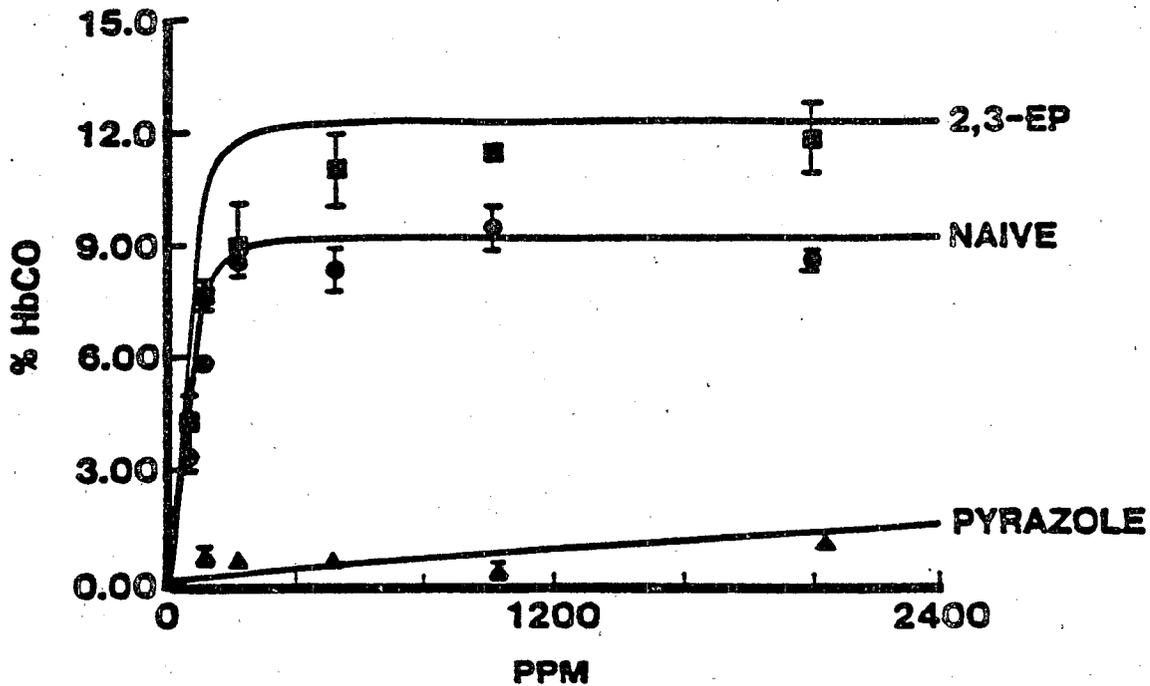


Figure 3. The end of exposure HbCO levels in naive, 2,3-EP, and pyrazole pretreated animals following 4-hour exposures to bromochloromethane. Three to nine animals were used for each exposure and the smooth curves were predicted by the computer model using the kinetic constants for bromochloromethane. The stoichiometric yield of CO from the oxidative pathway was 100% for 2,3-EP pretreated rats and was lowered to 70% for naive rats.

SOURCE: Gargas et al., 1986.

concentration in rats during exposure to DBM or concentration in rats during exposure to DBM or bromochloromethane (Figures 4 and 5) compared to the reduction in blood HbCO. Animals pretreated with 2,3-EP had only a minimal decrease in blood bromide compared to controls.

Gargas et al. (1986) concluded from their study that the effect of pyrazole pretreatment, which nearly abolished CO production, provides support for the involvement of cytochrome P-450 in the oxidation of DCM to CO. They also suggested that the metabolic intermediate, formyl chloride, produced during the oxidative metabolism of DCM, has a longer half-life in vivo than the formyl bromide intermediate produced during the oxidative metabolism of DBM. Therefore, "this would probably allow attack by a cellular nucleophile such as GSH [GTS], on the formyl chloride and would result in a reduced stoichiometric yield of CO in rats with normal GSH concentrations." Based on this line of reasoning, Gargas et al. (1986) suggested that a significant portion of the formyl chloride (20% to 30%) may react with other nucleophiles probably yielding CO₂.

Gargas et al. (1986) also reported that the experimental data did not show a stoichiometric relationship between the yield of CO and blood bromide in animals pretreated with 2,3-EP. They concluded from this observation that any alteration in the metabolic pathways (by inhibitors) must not compromise total metabolism (i.e., halide release). Thus, the kinetic constants calculated by the authors to assess in vivo metabolism were

CH₂BrCl

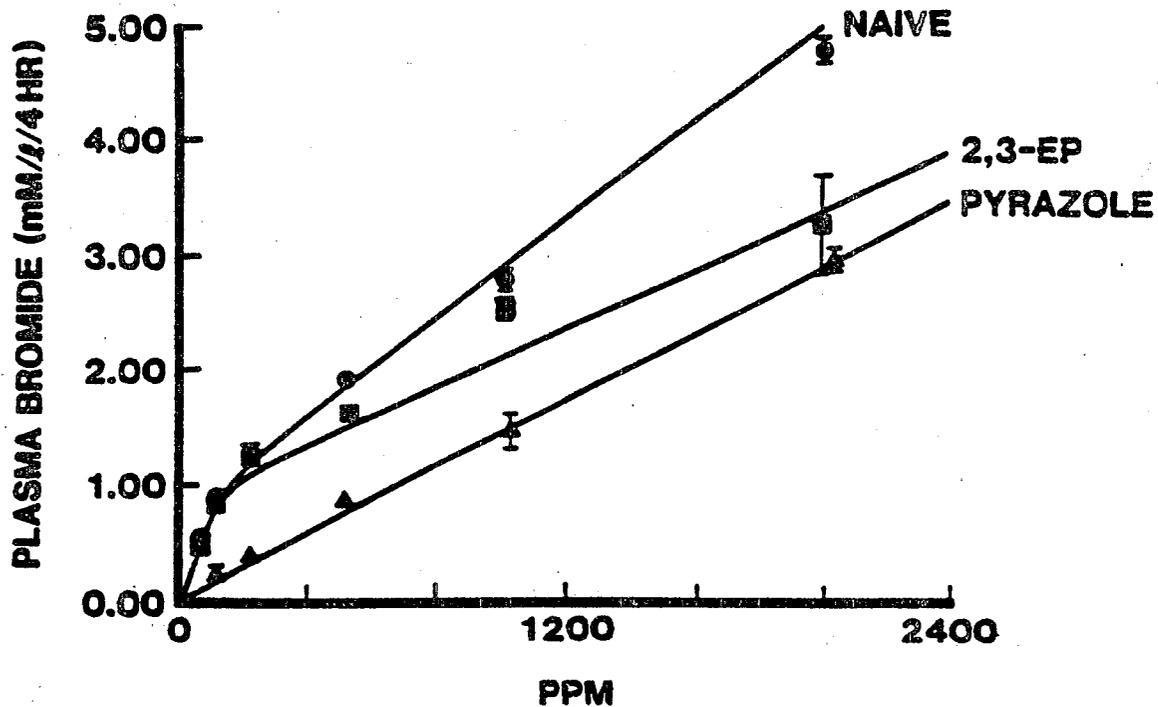


Figure 4. The dependence of plasma inorganic bromide levels on the ambient concentrations of bromochloromethane following 4-hour exposures using naive, 2,3-EP (O), and pyrazole (A) pretreated rats. Three to nine animals were used for each exposure and data are $\bar{X} \pm SE$ (error indicated by the vertical lines). Individual data points with no apparent error bars are those points where the SE is less than the size of the symbol. The smooth curves were generated by using the kinetic constants for bromochloromethane.

SOURCE: Gargas et al., 1986.

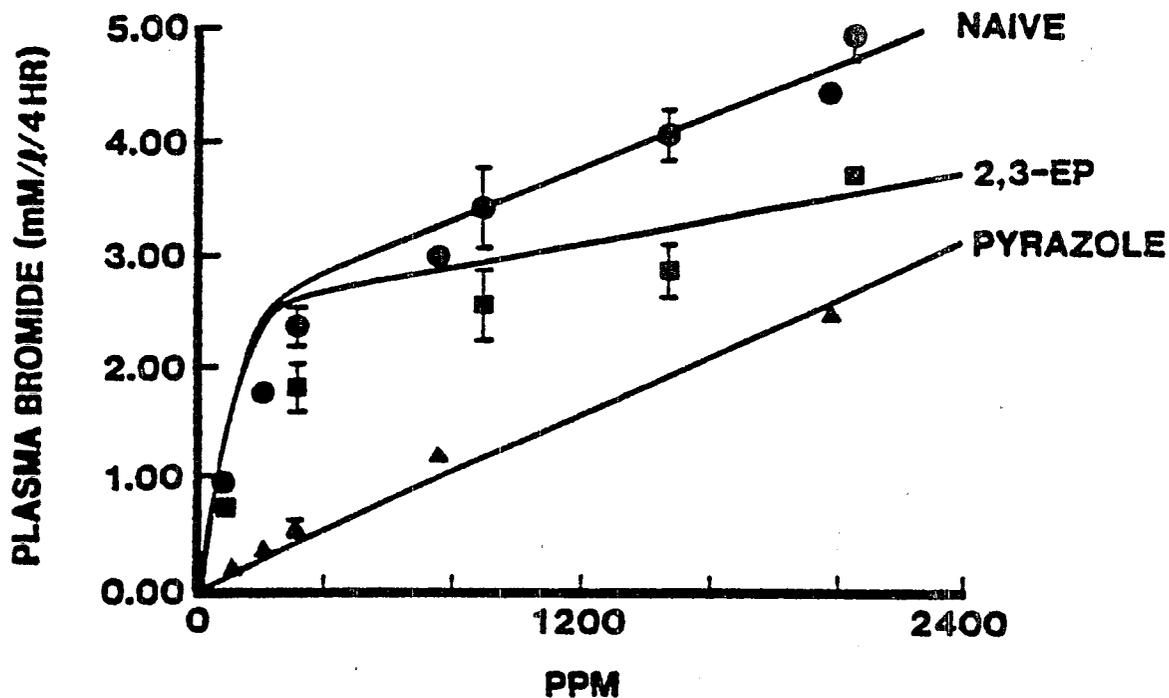
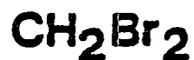


Figure 5. The dependence of plasma inorganic bromide levels on the ambient concentrations of dibromomethane following 4-hour exposures using naive, 2,3-EP, and pyrazole pretreated rats. Three to six animals were used for each exposure and the smooth curves were generated by the computer model using the kinetic constants for dibromomethane.

SOURCE: Gargas et al., 1986.

based on gas uptake and plasma bromide concentration. The calculated kinetic constants derived from the data obtained in these studies have led these investigators to label the oxidative pathway as "high affinity, low capacity" and the GST pathway as "low affinity, high capacity."

However, the lack of stoichiometry suggests an alternative explanation of the data obtained by Gargas et al. (1986). In animals pretreated with 2,3-EP, there is a significant increase in blood HbCO which could suggest an underutilization of the oxidative pathway. Thus, the lack of stoichiometry between CO production and blood bromide could be explained by a significant underestimation of the metabolism of DBM via the GST pathway. If this alternative explanation is correct, in control animals the CO pathway would appear to be saturated at very low concentrations and is consistent with the observed increase in blood HbCO in animals pretreated with 2,3-EP. Since Gargas et al. (1986) did not measure the metabolism of dihalomethanes to CO₂, it is not possible to directly estimate the metabolic activity attributed to the GST pathway nor the effect of 2,3-EP on metabolic activity attributed to that pathway.

Reitz et al. (1986) exposed groups of male B6C3F1 mice to either 50 or 1500 ppm ¹⁴C-DCM in order to study the in vivo conversion of DCM to ¹⁴CO and ¹⁴CO₂. Groups of four mice were exposed to ¹⁴C-DCM for 6 hours and ¹⁴CO, ¹⁴CO₂, and urine were collected during the exposure period and for 24 hours post-exposure. Reitz et al. (1986) compared control values to those

obtained from animals pretreated with pyrazole or pretreated with diethylmaleate and buthionine sulfoxime (BSO/DEM) (which depletes glutathione) in order to assess the relative contribution each pathway makes to the total metabolism of DCM. In animals pretreated with pyrazole, there was an apparent decreased amount of DCM metabolized to CO and CO₂ (Table 1). In animals pretreated with BSO/DEM, there was also an apparent decrease in the amount of DCM metabolism to CO and CO₂ (Table 1). However, BSO/DEM pretreatment resulted in a more significant decrease in DCM metabolism to CO₂ than CO. The authors interpreted the effect of pyrazole as showing that the microsomal oxidation of DCM yields both CO and CO₂. The authors did not offer an explanation for the decreased conversion of DCM to CO in animals pretreated with BSO/DEM, but did interpret the reduced conversion to CO₂ as supporting previous observations that DCM is metabolized by a system requiring glutathione.

TABLE 1. METABOLISM OF DCM TO CO AND CO₂ IN MICE

| | CO ^a | CO ₂ ^a |
|--------------------------|-----------------|------------------------------|
| <u>Exposure 50 ppm</u> | | |
| Control | 0.118 | 0.130 |
| BSO/DEM | 0.070 | 0.102 |
| Pyrazole | 0.040 | 0.062 |
| <u>Exposure 1500 ppm</u> | | |
| Control | 0.908 | 1.88 |
| BSO/DEM | 0.685 | 0.905 |
| Pyrazole | 0.125 | 0.565 |

^aValues are mM/kg.

SOURCE: Reitz et al., 1986.

A review of available data on the use of pyrazole as an in vivo inhibitor indicates that this compound affects a variety of metabolic systems including thyroid function and alcohol dehydrogenase activity (Szabo et al., 1978; Cornell et al., 1983). The observation that pyrazole inhibits alcohol dehydrogenase suggests that it might also inhibit the activity of formaldehyde dehydrogenase. At present, the available data do not exclude the possibility that pyrazole also inhibits the cytosolic metabolism of DCM to CO₂. Thus, it would seem premature, at this time, to attribute the observations of Reitz et al. (1986) to pyrazole inhibition of microsomal enzyme activity. The caution is especially applicable to the use of pyrazole as an inhibitor in the elucidation of in vivo DCM metabolism, since the available data suggest that a variety of physiologic functions are affected by pyrazole.

Reitz et al. (1986) also observed that animals pretreated with DEM (see Table 1), a known depletor of cellular glutathione, reduced the metabolism of DCM to both CO and CO₂. Reitz et al. (1986) did not offer an explanation for the reduced exhalation of CO in animals pretreated with DEM.

Stevens et al. (1980) demonstrated that rats pretreated with DEM show a significant decrease in the conversion of DBM to blood CO compared to controls. They also showed that the conversion of DBM to CO was significantly reduced in isolated hepatocytes and by isolated microsomes. These observations are consistent with the suggestion of Anders et al. (1978) that DEM may directly

inhibit certain cytochrome P-450 catalyzed oxidations. These data suggest that the attempt by Reitz et al. (1986) to characterize the in vivo metabolism of DCM by pretreating animals with various inhibitors will require revision.

Lastly, a search of the literature did yield data that would support the presence of significant amounts of GST activity associated with the microsomal fraction of the cell. Morgenstern et al. (1979) and Boyer et al. (1982) demonstrated that GST activities are associated with rat liver microsomes. Using 2,4-dinitrochlorobenzene as a substrate, the activity of the microsomal GST was 81 nmol/min/mg protein (Boyer et al., 1982). Boyer et al. (1982) also showed that the GST associated with the microsomal fraction of the liver could be activated increasing the specific activity to 1220 nmol/min/mg protein. This latter observation has suggested to Boyer et al. (1982) that the GST associated with the microsomal fraction of the liver could be an important detoxification mechanism. A search of the literature, however, did not yield information that demonstrated the use of this proposed detoxification mechanism. Thus, the available data supporting the hypothesis that microsomal oxidation of DCM yields significant amounts of CO₂ should be considered very limited at this time. Research will be required to reduce the uncertainties concerning the significances of the branch in the microsomal pathway as well as the characterization of the metabolic activity attributed to the proposed branch before it can be quantitatively factored into an analysis of DCM metabolism.

It is not clear from the hypothesis proposed by Gargas et al. (1986) why the glutathione intermediate produced by the microsomal pathway is significantly less reactive, and therefore less genotoxic, than the proposed glutathione intermediate in the cytosolic pathway. The intermediate proposed by Gargas et al. (1986) is similar in structure to the one proposed by Ahmed and Anders (1976) for the GST pathway and, therefore, could be expected to be equally toxic. Lastly, it should be noted that there are some data to indicate that the metabolic intermediate(s) produced by the microsomal pathway is mutagenic (Jongen et al., 1982) and, therefore, probably genotoxic.

CEFIC (1986f) studied the in vivo metabolism of ^{14}C -DCM in rats and mice. Groups of three animals were exposed to 500, 1000, 2000, or 4000 ppm DCM for 6 hours. These investigators monitored blood levels of the parent compound and HbCO during and after the exposure period. In addition, they monitored exhalation of ^{14}CO and $^{14}\text{CO}_2$ postexposure. In rats, the blood level of DCM is proportional to the dose at exposures above 500 ppm. In mice, the blood level of the parent compound is not proportional to dose until exposures reached 2000 ppm. The authors interpreted this observation as a difference in the metabolic capacity of the species, i.e., mice can metabolize significantly more DCM via the GST pathway than rats. The data on blood levels of DCM in rats supports the conclusion that there is a dose-response relationship between blood levels and exposure above 500 ppm. However, the data from the mouse studies are

highly variable which suggests that conclusions based on average values used by CEFIC (1986f) must be viewed with caution. For example, the blood level of DCM in mice exposed to 2000 ppm varied from 40 ug/mL at 1.5 hours to 20 ug/mL at 6 hours.

Following exposure to 4000 ppm DCM, mice exhale more CO₂ than rats. Based on this observation, CEFIC (1986f) concluded that mice metabolize more DCM via the GST pathway than rats. The authors used differences in blood levels of the parent compound as a surrogate to suggest that there is a species difference in DCM metabolized to CO₂ during exposure. The data obtained by CEFIC (1986f) are significantly different from those found in the published literature. McKenna et al. (1982) exposed groups of rats to 50, 500, or 1500 ppm and found little differences in the percent conversion of DCM to CO and CO₂ at each dose over the exposure range tested. Angelo et al. (1984) exposed mice or rats to either 10 or 50 mg/kg DCM and clearly demonstrated that the percent conversion to CO and CO₂ were similar at both dose levels and in both species. The McKenna et al. (1982) and Angelo et al. (1984) studies question correctness of the assumption made by CEFIC (1986f) that the blood level of the parent compound is an appropriate surrogate for metabolism of DCM to CO₂.

3.2. REACTIVE INTERMEDIATES

In the elucidation of DCM metabolism, Ahmed and Anders (1976) and Kubic and Anders (1978) predicted formation of a reactive intermediate by both the microsomal and cytosolic pathways. Andersen et al. (1986, 1987), however, suggested that

the metabolism of DCM via the microsomal pathway does not result in the formation of a reactive intermediate. These investigators cite two observations to support this hypothesis. First, they suggest that the lack of tumor development in the drinking water study (National Coffee Association, 1982a, b; 1983) can be explained by assuming that at the low doses all DCM is metabolized via the microsomal pathway. It should be noted, however, that the Carcinogen Assessment Group (U.S. EPA, 1985a, b) predicted, based on the tumor response in the National Toxicology Program (NTP) study, that there would not be a significant tumor response in the drinking water study without invoking differences in the utilization of metabolic pathways.

The second observation cited by Andersen et al. (1986, 1987), that the metabolism of DCM via the microsomal pathway does not result in the formation of a reactive intermediate, comes from the work of Green (1983). Green (1983) investigated the mutagenic potential of DCM and chlorofluoromethane (CFM) using Salmonella typhimurium strain TA100 in an Ames type assay. S. typhimurium exposed to either DCM or CFM gave a mutagenic response without the addition of rat liver fractions. The addition of rat liver post-mitochondrial supernatant (S9) did not significantly increase the mutagenic response of TA100 exposed to DCM, but did increase the mutagenic response of TA100 exposed to CFM. Green (1983) also observed that the addition of microsomes did not increase the mutagenic response of TA100 exposed to DCM, but the addition of cytosol did increase the mutagenic response

(Table 2). He concluded from these observations that the lack of mutagenic response by the intermediate generated by microsomal metabolism could be explained by the fact that the reactive intermediate was highly unstable (exists at -60°C in inert solvents).

TABLE 2. THE MUTAGENIC EFFECT OF DIHALOMETHANES

| Addition | DCM ^a | CFM ^a |
|-------------------------|-----------------------------|-----------------------------|
| None | 386 ± 24 | 521 ± 27 |
| S9 | 458 ± 24 | 793 ± 17 |
| Cytosol ^b | 490 ± 19 (698) ^c | 618 ± 14 (812) ^c |
| Microsomes ^b | 375 ± 31 | 623 ± 27 |
| Boiled S9 | 376 ± 28 | |
| Air control | 80 ± 1 | |

^aValues are revertants/plate ± SD after 3-day exposure.

^bCytosol and microsomal concentrations (mg protein) are equivalent to those in the S9/cofactor mixture.

^cCytosol concentration increased threefold.

SOURCE: Adapted from Green, 1983.

However, the inability of Green (1983) to demonstrate a mutagenic response by the reactive intermediate generated during microsomal oxidation of DCM may be the result of experimental design, specifically the long incubation period. Green (1983) carried out incubations for 3 days while other investigators, performing similar types of experiments, have used much shorter incubation periods. Van Bladeren et al. (1980) studied the mutagenic response of *S. typhimurium* TA100 exposed to dibromo- or diiodomethane. These investigators, using a 15-minute incubation

period, clearly showed that the addition of microsomes or cytosol markedly increased the number of revertants/plate using either dibromo- or diiodomethane as a substrate. Similarly, Jongen et al. (1982), using a 6-hour incubation period, also demonstrated that the addition of microsomes or cytosol increased the number of revertants/plate of S. typhimurium exposed to DCM (Table 3).

TABLE 3. THE MUTAGENIC EFFECT OF DCM ON S. TYPHIMURIUM TA100 IN THE PRESENCE OF VARIOUS RAT LIVER FRACTIONS

| Addition | No. of Revertants ^a |
|----------------------|--------------------------------|
| <u>Aroclor</u> | |
| Control ^b | 939 |
| Microsomes | 1201 |
| Cytosol | 1407 |
| Cytosol+DEM | 889 |
| <u>Phenobarbital</u> | |
| Control | 540 |
| Microsomes | 624 |
| Cytosol | 806 |
| Cytosol+DEM | 567 |

^aNet number of revertants of three plates.

^bControl equals microsomes minus cofactors.

SOURCE: Adapted from Jongen et al., 1982.

Using an Ames bioassay, the available data on the formation of reactive intermediates are limited to a few studies. All studies reported to date have shown that the metabolism of DCM using a liver cytosolic fraction leads to formation of a reactive intermediate. The various studies, taken together, suggest, but

do not prove, that the metabolism of dihalomethanes via microsomal oxidation lead to formation of reactive intermediates. The data also suggest that the reactivity of the intermediates formed using different dihalomethanes as substrates may not be equal. Regarding the metabolism of DCM, the data from some studies suggest that the metabolism of DCM by the microsomal cytochrome P-450 pathway leads to formation of a reactive intermediate. Further research is required to resolve the lack of agreement among investigators on formation of a reactive intermediate during microsomal metabolism of DCM.

3.3. USE OF IN VITRO DATA TO PREDICT IN VIVO METABOLISM

Gargas et al. (1986) characterized the microsomal metabolism of DCM as "high affinity - low capacity" and the cytosolic metabolism as "low affinity - high capacity." The data used to support this characterization comes from the observation that the microsomal enzyme has a much lower K_M (Table 4) than the cytosolic enzyme (Table 5).

TABLE 4. KINETIC CONSTANTS FOR THE METABOLISM OF DCM TO CARBON MONOXIDE BY LIVER MICROSOMES

| Species | K_M (mM) | V_{max} (nmol/min/mg protein) |
|---------|---------------|---------------------------------------|
| Mouse | 0.79 | 1.94 |
| Rat | 0.86 | 0.58 |
| Hamster | 2.83 | 1.85 |

SOURCE: Adapted from CEFIC, 1986e.

TABLE 5. KINETIC CONSTANTS FOR THE METABOLISM OF DCM TO FORMALDEHYDE BY LIVER CYTOSOL

| Species | K_M (mM) | V_{max} (nmoles/min/mg protein) |
|---------|--------------------|--------------------------------------|
| Mouse | 86 | 36.4 |
| Rat | 21 | 2.9 |
| Hamster | No detectable rate | |
| Man | No detectable rate | |

SOURCE: Adapted from CEFIC, 1986e.

However, the K_M and V_{max} values reported by CEFIC (1986e) (1986a) are significantly different from those published by Ahmed and Anders (1976) and Anders et al. (1978). These investigators obtained K_M and V_{max} values of 50.1 mM and 5.4 nmol CO/min/mg protein for the metabolism of ^{14}C -DCM to CO using a rat liver microsomal preparation (Table 6). They also reported K_M and V_{max} values of 48 mM and 16 nmol formaldehyde/min/mg protein for the metabolism of DBM by a rat liver cytosolic preparation.

Thus, the K_M and V_{max} values obtained by Ahmed and Anders (1976) and Anders et al. (1978) do not support the conclusion by Gargas et al. (1986) that the microsomal pathway should be considered "high affinity - low capacity" and the cytosolic pathway should be considered "low affinity - high capacity." The difference in the K_M value of the microsomal/cytosolic pathway is 1/23 according to the data reported by CEFIC (1986e) but only 1/2.4 based on the data from Ahmed and Anders (1976) and Anders et al. (1978). Furthermore, the K_M and V_{max} values obtained by

TABLE 6. KINETIC CONSTANTS FOR THE METABOLISM OF DIHALOMETHANES TO CARBON MONOXIDE BY RAT LIVER MICROSOMES

| Author | K_M (mM) | V_{max} (nmoles/min/mg protein) | Substrate |
|-------------------------|---------------|---|-----------|
| CEFIC (1986e) | 0.86 | 0.58 | DCM |
| Anders et al. (1978) | 50.1 ± 5.2 | 5.4 ± 1.7 | DCM |
| Ahmed and Anders (1976) | 48.1 ± 6.2 | 15.5 ± 2.5 | DBM |

Ahmed and Anders (1976) are consistent with the in vivo observations made by McKenna et al. (1982) and Angelo et al. (1984) that over a wide range of doses there is little difference in the percentage of DCM metabolized to CO and CO₂.

In addition, neither Gargas et al. (1986) nor CEFIC (1986e) included in their in vitro to in vivo extrapolation a consideration of differences in total enzyme in the tissue nor did they account for the effect of cellular architecture on the distribution of DCM within the cell. The available data suggest that DCM is taken-up by cells via passive diffusion. Thus, given our current understanding of cellular architecture, it can be assumed that both the endoplasmic reticulum (ER) (microsomes) and the cytosolic fraction of the cell will be exposed equally to DCM. Since the cytosolic fraction of the cell occupies significantly more volume than the ER, one could reasonably predict that a significant amount of DCM would be available for metabolism by the cytosolic enzyme. In addition, on a mass basis, the microsomal fraction of the cell is about 2% to 5%

while the cytosolic fraction of the cell is about 10%.

Consistent with this analysis is the observation that animals exposed to low concentrations of DCM exhale significant amounts of CO₂ (McKenna et al., 1982; Angelo et al., 1984; Reitz et al., 1986). Furthermore, this analysis is also consistent with the observation that animals exposed to high concentrations of DCM exhale significantly more CO₂ than CO.

3.4. IN VITRO METABOLISM

Gargas et al. (1986) predicted that microsomal metabolism of DCM might yield both CO and CO₂. The Gargas et al. (1986) hypothesis suggests that microsomal metabolism of DCM proceeds via the mechanism outlined in Figure 6. The mechanism predicts that following the initial oxidation of DCM to formyl chloride, this intermediate combines with glutathione and is then further metabolized by a GST to CO₂. Gargas et al. (1986) suggested that the formation of the formyl glutathione intermediate is possible assuming that formyl chloride is a relatively stable intermediate. The assumption that the formyl chloride is relatively stable, thus allowing for formation of the formyl glutathione, is in marked contrast to the observation made by Green (1983) that formyl chloride was nonmutagenic because it was such an unstable intermediate. The only data supporting this prediction is the observation by Reitz et al. (1986) that mice pretreated with pyrazole exhale less CO and CO₂.

CEFIC (1986e) recently submitted to the HRAC information on the in vitro metabolism of DCM in tissues from the rat, mouse,

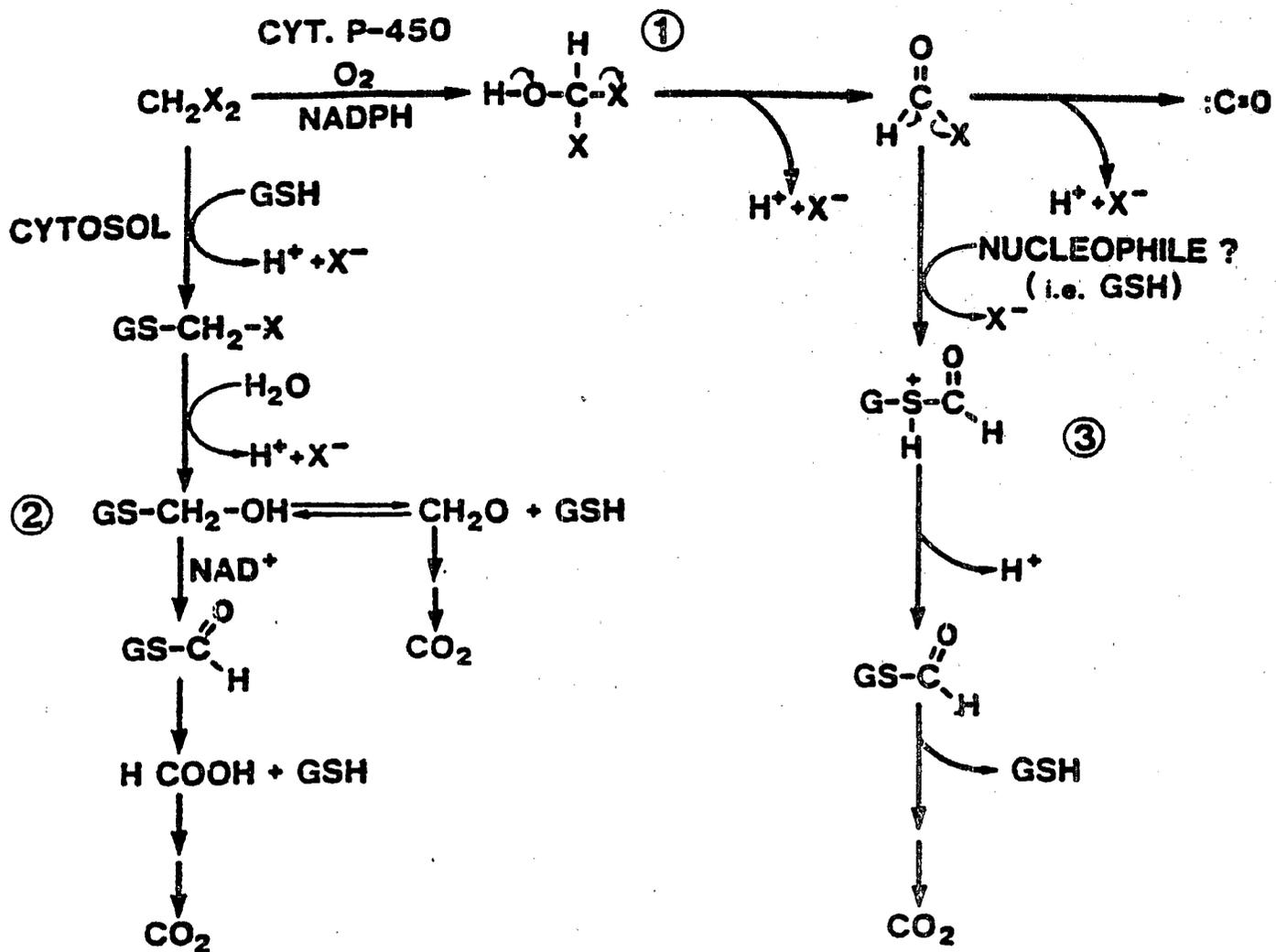


Figure 6. The proposed pathways for dihalomethane metabolism. Pathways 1 and 2 were taken from Anders et al. (1977) and pathway 3 is proposed by Gargas et al. (1986).

SOURCE: Gargas et al., 1986.

hamster, and human. These investigators measured the conversion of DCM to CO using microsomal preparations from lung and liver tissue and the conversion of DCM to formaldehyde by the cytosolic fraction from lung and liver tissue. In addition, they measured the cytochrome P-450 content of lung and liver microsomes and the GST activity in the cytosolic fraction of lung and liver tissue using 2,4-dinitrochlorobenzene as a substrate.

CEFIC (1986e) reported that the highest microsomal-specific activities (DCM converted to nmol CO/mg min/protein) were measured in hamster liver followed by mouse lung and liver. The specific activity of rat liver microsomes was about one-third that of mouse liver. The single measurement for human liver microsomal metabolism of DCM to CO was 150% of the value obtained for rat liver. The authors state, based on this single measurement, that the value for human liver microsomal metabolism of DCM to CO was similar to that for rat liver. Mouse, rat, and hamster lung microsomal preparations were also assayed for DCM metabolism to CO. Mouse lung microsomes had the highest activity, hamster next, and rat the lowest, having about one-tenth the activity of mouse lung microsomes. Human lung microsomes were not assayed for DCM metabolic activity.

The in vitro data presented on DCM metabolism to CO suggests that there are significant metabolic differences between species (CEFIC, 1986e). Unfortunately, these investigators did not carry out experiments that allowed for determining the stoichiometric relationship between the amount of DCM metabolized to the amount

of CO produced. The lack of stoichiometric measurements does not allow one to determine if DCM metabolism to other end products such as CO₂ could explain the apparent species differences. Indeed, if the hypothesis put forth by Gargas et al. (1986) and the data from Andersen et al. (1986, 1987) and Reitz et al. (1986) are correct, then one could expect formation of significant amounts of other metabolites during DCM metabolism. Lastly, the cytochrome P-450 content of lung and liver microsomes reported by CEFIC (1986e) differ from those previously reported in the literature (Souhaili-El Amri et al., 1986). The differences are small, less than 20% for rat liver and 168% for mouse liver, but large for human liver, 368%. The significance of these differences and the correlation between cytochrome P-450 content and DCM metabolism will require additional research.

CEFIC (1986e) assayed mouse, rat, hamster, and human liver cytosolic fractions for the metabolism of DCM to formaldehyde by the GST pathway. Mouse and rat liver, but not hamster or human liver, had detectable levels of DCM metabolized to formaldehyde. In addition, mouse, but not rat or hamster, lung cytosolic fractions had detectable levels of DCM metabolized to formaldehyde. The investigators concluded from these observations that the presence of DCM metabolism to formaldehyde is consistent with the tumorigenic response in the mouse lung and liver and the lack of tumorigenic response in the rat and hamster. However, a review of the methodologic approach used to assay for DCM metabolism in the cytosolic fraction raises a

number of concerns. First, it is not clear why these investigators decided to measure only formaldehyde. The approach used presents a number of variables for which control data have not been reported. These include: species differences in the metabolism of formaldehyde, and stoichiometry of the reaction. Indeed, the lack of detectable levels of formaldehyde might readily be explained by the large excess of formaldehyde dehydrogenase found in both lung and liver tissues (Uotila and Kiovusalo, 1981). Also, it is not clear why these investigators selected the Nash (1953) method for assaying for formaldehyde formation. The method is not specific for formaldehyde and gives a high background level because of its reactivity with a variety of substrates.

Ahmed and Anders (1976) also used the Nash method to demonstrate the metabolism of dihalomethane (DBM) to formaldehyde by rat liver cytosol. These investigators, however, reported values significantly greater than those reported by CEFIC (1986e). Ahmed and Anders (1976), unlike CEFIC (1986e), dialyzed the cytosolic preparation before conducting the assay and found that the rate of formation of formaldehyde was three times greater for dialyzed cytosol compared to undialyzed. These investigators also could not demonstrate a stoichiometric relationship between bromide release and formaldehyde formation with the observed concentrations of free bromide being about twice the predicted based on the observed concentration of formaldehyde. The values reported by Ahmed and Anders (1976)

suggest that formaldehyde formation may have been underestimated.

CEFIC (1986e) also assayed liver and lung cytosolic fractions for GST activity using 2,4-dinitro-chlorobenzene as a substrate. The values reported by CEFIC (1986e) for 2,4-dinitrochlorobenzene metabolism in all tissues assayed were significantly smaller than those reported by other investigators (Lorenz et al., 1984). The difference for all tissues was about 40% of the value reported by Lorenz et al. (1984) but similar to the value reported by Moron et al. (1979) for rat liver cytosol. However, both Lorenz et al. (1984) and Moron et al. (1979) reported similar and significantly larger values than CEFIC (1986e) for rat lung GST using 2,4-dinitrochlorobenzene as a substrate (Table 7). Since stability of enzymes can vary, it may be necessary to determine if there are changes in the ability of different tissues to metabolize DCM when stored for various periods of time.

The methodology used by CEFIC (1986e) indicates that the amount of protein used to assay for enzymatic activity varied significantly from tissue to tissue and species to species. The difference in protein concentration may significantly affect the amount of metabolic end product measured since the reactive intermediates formed during the metabolism of DCM bind to protein and lipid (Anders et al., 1977). In addition, because of the differences in protein concentration, it is not clear that the glutathione concentrations were optimal in all experiments. Thus, in the absence of stoichiometric data and control

TABLE 7. THE METABOLISM OF 2,4-DINITROCHLOROBENZENE BY RAT LIVER AND LUNG CYTOSOL

| Author | Liver ^a | Lung ^a |
|----------------------|--------------------|-------------------|
| CEFIC (1986e) | 504.9 | 44.2 |
| Lorenz et al. (1984) | 1380 ± 110 | 77.5 |
| Moron et al. (1979) | 567 ± 184 | 99.8 ± 11.4 |

^aValues are nmol/min/mg protein.

experiments, it is not possible to determine if the experiments were carried out under optimal conditions nor if the values reported represent total metabolism. Lastly, it should be noted that the values reported by CEFIC (1986e) represent a single experiment using pooled tissue samples. If one is to conclude that there are significant species differences in the metabolism of DCM, then it will be necessary to expand the data base to demonstrate statistical significance.

The in vitro data reported by CEFIC (1986e) may allow for alternative approaches to interspecies extrapolation in assessing the effects of exposure to DCM. However, before such extrapolations are useful, the uncertainties raised about the quality of the data reported need to be resolved. In addition, it would be useful to expand the human tissue data base beyond a single data point.

3.5. SUMMARY

The recent reports by Gargas et al. (1986), CEFIC (1986e, f), and Reitz et al. (1986) have raised a number of potential

issues regarding species differences in the metabolism of DCM which have suggested to these investigators alternative approaches for assessing human risk from exposure to DCM. The hypothesis put forth by these investigators is supported by very preliminary data. An analysis of the data suggests that there is a need to conduct critical experiments to support the hypothesis as well as to rule out alternative explanations. In addition, there is a need to conduct experiments to help explain why some of the observations made by these investigators are significantly different from those found in the published literature.

The hypothesis that microsomal oxidation of DCM leads to formation of significant amounts of CO₂ is based almost entirely on a series of assumptions and some indirect measurements. Therefore, one must be very cautious in accepting the conclusion that microsomal oxidation of DCM leads to CO₂ formation given the lack of data to support similar types of metabolism by microsomes.

Gargas et al. (1986) and CEFIC (1986e), using in vitro enzyme kinetic values, have made the assumption that at low doses DCM is almost exclusively metabolized in vivo by the microsomal pathway. The appropriateness of this extrapolation is based almost exclusively on indirect measurements and a series of unvalidated assumptions. At present, the available data do not allow for distinguishing differences in the utilization of the microsomal and cytosolic pathways at low versus high doses of DCM

in in vivo metabolism. First, there is a significant body of data in the published literature which strongly suggests that both the microsomal and cytosolic studies by Gargas et al. (1986) can be interpreted in a way which would strongly support the assumption that at low doses significant metabolism of DCM via the cytosolic pathway occurs in vivo. Thus, at present, data are lacking to support the assumption that, at low doses, DCM is almost exclusively metabolized via the microsomal pathway.

The data supporting possible differences in the characterization of the reactive metabolic intermediates formed by microsomal and cytosolic metabolism of DCM are limited. The available data suggests that further characterization of the reactive intermediates may be useful to better delineate species differences in the metabolism of DCM.

The observation by CEFIC (1986e) that human tissue does not metabolize DCM by the cytosolic pathway must be viewed with a great deal of reservation. The observation is based on a single sample of tissue, and control experiments indicate metabolic activity that is significantly less than reported by others (Lorenz et al., 1984). The CEFIC (1986e) study, however, does suggest that development of a data base that includes metabolic activity in human tissues could substantially reduce the uncertainties in our present risk assessment.

4. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL: METABOLIC KINETIC CONSTANTS

Some of the critical determinations for the use of the physiologically based pharmacokinetic (PBPK) model used by Andersen et al. (1986, 1987) (Chapter 2) are the values of the kinetic constants (k_F , V_{max} , K_M) for the metabolism of DCM and how these are related among the various species. The following discussion makes the assumption that the model is valid (which is not necessarily true, as discussed in Chapter 2). This assumption includes an important provision relating to the kinetic constants, that is, that k_F is the constant relating to a pathway that involves GST, and that this pathway is nonsaturable (therefore needing to be defined by only one constant). V_{max} and K_M , on the other hand, are standard kinetic constants which apply to a second, saturable mixed-function oxidase (MFO) pathway. Classically, the GST pathway leads to carbon dioxide and the MFO pathway leads to carbon monoxide as end products, using DCM as a substrate.

Originally, these kinetic constants were based on the specific activities of GST and monooxygenase measured in samples of lung and liver tissue from four species by Lorenz et al. (1984) using 1-chloro-2,4-dinitrobenzene (for GST) and 7-ethoxycoumarin (for monooxygenase) as substrates. Since these critical constants were not based on experimentation using DCM itself as a substrate, the assumption that the Lorenz et al. data

served as an appropriate substitute was tenuous.

In a subsequent version of the model used by Andersen et al. (1986, 1987) used a curve-fitting exercise to obtain the "whole tissue" values of the three kinetic constants in animals. With all other physiological and biological parameters kept constant, the values of K_M , V_{max} , and k_F were varied in an intricate computer optimization procedure. The pharmacokinetic model was used to arrive at predictions that best approximate the actual experimental chamber data in which disappearance of DCM over time was monitored.

For humans, values of K_M (0.58 mg/L) and V_{max} (119 mg/hr) for the oxidative pathway were estimated (Andersen et al., 1987) from unpublished human experimental data in which DCM concentrations in expired air were measured following exposure by inhalation. For the critical GST pathway, however, no human data were available upon which an estimate of k_F can be based. The authors noted that allometric scaling, based on the concept of clearance, served to adequately relate the mouse, hamster, and rat data, using a factor of body weight to the 0.7 power. Using this scaling procedure, the authors obtained a human value of 0.53 hr^{-1} for k_F . This value, by the authors' methodology, leads to a reduction in risk of somewhat less than an order of magnitude, as compared to risks calculated without incorporating species-to-species and high- to low-dose extrapolative pharmacokinetic information (all other risk assessment assumptions being unchanged). Also, the value results from

assuming that (1) the GST pathway is the only toxic pathway, and (2) carcinogen risk is directly related to concentration at a "target" site.

If other combinations of K_M , V_{max} , and k_F could be found which, when put into the pharmacokinetic model, approximate the experimental concentration versus time curves to an extent similar to that found by the authors' optimization scheme, then the scaling approach would be suspect. This is the case, in fact; values of k_F , which vary as much as fourfold from the optimized values, were used in the model, leading to alternative combinations that fit the experimental data nearly as well as the optimized values.

It is therefore concluded that, although the optimization procedure may lead to kinetic constants giving an optimal fit (using the pharmacokinetic model) to the experimental chamber data, there are alternative combinations of the three kinetic constants that can virtually do the same thing. This may be due, perhaps, to the fact that the "optimized" solution may have a goodness-of-fit that only trivially exceeds those from other fits having substantial differences in the underlying constants. In other words, there are multiple adequate solutions to the problem (redundancy). On this basis, the scaling approach for the determination of a human k_F value, a critical value with regard to DCM carcinogenicity given that the model used by Andersen et al. (1986, 1987) is valid, is subject to uncertainties which render the approach suspect. Thus, the use of the scaling

approach is still in the preliminary stages of development, and more research and validation is required before it can be accepted as a reasonable basis for pharmacokinetic modeling in the case of DCM.

New data have become available subsequent to the proposal of a pharmacokinetic model for DCM by Andersen et al. (1986, 1987). These experiments have the potential to measure the human GST pathway; thus, other approaches for obtaining human GST data, such as the scaling approach described above, would not be necessary. The data released by ICI-UK (CEFIC, 1986e, f) are interpreted by those investigators as indicating that there is little or no risk of cancer to humans as a result of exposure to DCM. The cornerstone of this argument is the same as proposed by Andersen et al. (1986, 1987)--that the toxic intermediate is produced only by the GST pathway. The results of this study (CEFIC, 1986e, f) are interpreted as showing that there is no GST activity in humans with regard to DCM, as opposed to mice, where such activity is easily detected.

CEFIC's (1986e) critical experiment used human liver derived from accident victims. Fractions of the liver, dosed with DCM, were assayed for formation of formaldehyde. The amount of formaldehyde produced was considered to be a measure of the fraction's GST activity towards DCM. No excess (over background) of formaldehyde was found at any dose of DCM. In livers derived from mice, a dose-related formation of formaldehyde was observed.

If humans indeed have no GST pathway for DCM, k_F (as

described above) would be zero, and there would be no risk if this were the only toxic pathway. There are, however, many problems associated with the CEFIC (1986e) experimentation; some of the more important ones are briefly discussed here. First, CEFIC (1986e) did not present information regarding whether or not the critical control experiment was done. That is, adding a known amount of formaldehyde (for example, the amount at the limit of detection) to the reaction mixture and seeing if this amount could be recovered by CEFIC's (1986e) assay procedure. This information is needed because formaldehyde is very reactive; it can bind to many subcellular fractions or enter other metabolic pathways. Small amounts actually generated by human liver could go undetected. [In April 1987, Harris of ICI-UK informed the HRAC by telegram that this experiment had been performed and that "less than 10% of the formaldehyde was lost over the incubation period." Details of the procedure, including the dose of formaldehyde, and results have not be provided.]

Second, it is difficult to calculate the maximum velocity at which human liver could metabolize DCM by the GST pathway. The calculation by CEFIC (1986e) suggests that the metabolic capability of the human liver GST could be anywhere from zero to one-sixtieth the mouse value (on a nmol/min/mg protein basis) based on the limit of detection. [Other preliminary calculations suggest that the mouse value may be even closer to the human value based on the data reported by CEFIC (1986e).] One-eighth of the mouse value is the relationship suggested by Andersen et

al. (1986, 1987) based on the scaling approach discussed above. Obviously, the uncertainty encompasses orders of magnitude.

Third, there is some question regarding the nature of the human liver samples used. That is, whether the events that occurred between the time that the liver was operating normally in the human and the time of the GST assay somehow affect whether or not GST activity would be detected (for example, what happens to the liver between the time of an accident and the time it is removed from the body). Such factors include introduction of drugs, and how the liver was maintained.

Fourth, there is difficulty in using the CEFIC (1986e) data in the model used by Andersen et al. (1986, 1987). Additional information is needed on the relationship between activity per amount of protein in the CEFIC (1986e) in vitro assays and how much of this activity would be in the entire liver. Assumptions can be made in this area, but additional uncertainty is the result.

Fifth, a number of incidental questions have not been answered, such as where the carbon dioxide comes from in vivo experiments using rats and hamsters treated with DCM if the GST pathway is negligible or absent using DCM as a substrate, as indicated from the CEFIC (1986f) analyses in these species. One hypothesis is that the MFO pathway can also produce carbon dioxide; the CEFIC (1986f) data show that all species tested, including humans, have MFO activity. However, this is only a hypothesis that may be questioned, as discussed previously.

Another question is whether or not other fractions of the liver homogenate, not just the cytosol, were assayed for DCM GST activity. Although this activity might be expected to be in the cytosol, other possibilities should not be ruled out on the basis of what is expected.

Sixth, CEFIC (1986e) only assayed human liver GST at a single pH value (pH 7.4) using DCM as a substrate. Since individual isozymes may have sharp pH optima in the acidic, neutral, or alkaline range, the CEFIC (1986e) assay may have selected against the detection of a human DCM-specific GST isozyme. Furthermore, it has been reported by Seidegard and Pero (1985) and Seidegard et al. (1985, 1986) that some humans (as many as 50% of the population) lack a specific principal GST isozyme that is present in the rest of the population. Thus, if one or more of the particular subjects studied by CEFIC (1986e) lacked a potential DCM specific GST isozyme, there would be a decreased sensitivity, or an inability to detect activity towards this substrate.

Finally, and of great importance, only human liver was assayed by CEFIC (1986e). Human lung, or other potential target organs such as the pancreas, were not assayed. If the premise of no human risk by virtue of the lack of metabolic capability by the GST pathway is to be assumed, then information from the liver only is not adequate to rule this metabolic capability out for the entire body.

In conclusion, there are many ways to calculate the kinetic

input into a pharmacokinetic model for DCM in order to estimate "target site" dose. The ultimate effect on estimates of human risk, using Andersen et al.'s methodologies and assumptions (which include that the GST pathway is the only toxic pathway, and that risks are related to concentration at a "target site"), ranges from a finding of little difference to a finding of no human risk at all from the traditional "applied dose" procedure. Additional investigations* (some of which are currently ongoing by ICI-UK and NTP as outlined in Chapter 2) and more data will be needed to narrow this large range of uncertainty.

*On May 26, 1987, HRAC members received a letter from Dr. R. Reitz of Dow Chemical Company and Dr. T. Green of ICI-UK indicating that both labs were able to find human GST activity using ^{36}Cl -DCM. Further data will be forthcoming on these findings. The ICI-UK lab, directed by Dr. Green, is the same lab that conducted the previously cited studies by CEFIC (1986a, b, c, d, e, f).

**5. MUTAGENICITY ASSESSMENT OF DICHLOROMETHANE:
REVIEW OF STUDIES PERFORMED BY CEFIC**

5.1. PHASE I TESTS

In Phase I of its investigation into the carcinogenic mechanism of DCM, CEFIC (1986b, c) tested the effect of the compound on rats and mice in two genotoxic assays: unscheduled DNA synthesis (UDS) in vivo and in vitro, and covalent binding to DNA in vivo.

5.1.1. In vivo Unscheduled DNA Synthesis

DCM was tested for its ability to induce DNA repair synthesis in liver cells of Fischer 344 rats and B6C3F1 mice treated in vivo (CEFIC, 1986b). Animals were exposed by inhalation to DCM at 2000 and 4000 ppm for either 2 or 6 hours. At the end of the exposure period, animals were perfused to generate hepatocyte cell suspensions which were then placed into culture. After allowing up to 2.5 hours for cell attachment, tritiated thymidine was added to the culture medium. After an additional 4 hours of incubation with fresh medium, the cultures were washed and incubated overnight with medium containing no label. The following day, the cells were fixed, dried, and prepared for autoradiography. Those cells that were undergoing "long patch" DNA repair during the 4-hour incubation period would be expected to show higher than background levels of exposed silver grains over hepatic nuclei.

There are several serious weaknesses in this study which

cast into doubt the usefulness of the results. First, only male animals were tested and no reason was given for not testing females. Second, the assay of in vivo UDS has inherent theoretical difficulties. Its use, rather than an in vitro method, is only justified in situations where extrahepatic metabolism may be a factor. Even then, this assay should be used only when metabolites generated in other tissues are sufficiently stable to travel to the liver and generate DNA damage in hepatocytes. A serious weakness of this assay is the relatively long period of time between agent administration and the assay of DNA repair. Excision repair presumably begins shortly after exposure to the test chemical. Before UDS can be measured, however, the animal first must be perfused, and then the hepatocytes isolated, placed into culture, and allowed to attach (the final step alone requiring approximately 2 hours). It has not been shown that UDS continues for a sufficient period of time to be reliably detected in vitro. These uncertainties are exacerbated further by the nature of the positive controls. A convention has developed that the positive control animals in this assay do not have to be similarly exposed in vivo (Mirsalis et al., 1980). Instead, hepatocytes isolated from untreated animals are exposed in vitro to diethylnitrosamine. In the opinion of the HRAC, this does not constitute an adequate positive control.

The third weakness of this study relates to dose selection. The authors selected the dosing regimens in order to deliver

doses equivalent to those used in the NTP bioassay. In effect, the investigators chose a dose normally used in a chronic study for this acute assay. This, of course, nearly guarantees a negative result for this study. Exposure levels for a lifetime bioassay are selected so that animals can be chronically dosed with sufficient numbers of animals surviving to the end of the study. Indeed, in the NTP study, the animals were given 2000 and 4000 ppm for 6 hours per day, 5 days per week, for 102 weeks. For an acute study such as the in vivo UDS, much higher doses should have been selected.

5.1.2. In vitro Unscheduled DNA Synthesis

For the in vitro UDS studies, hepatocyte cultures derived from untreated rats and mice were exposed to 500, 1000, 2000, and 4000 ppm DCM for 8 hours (CEFIC 1986b). The methods used in this study are similar to those described for the in vivo study except of course that dosing occurred in vitro. Dose selection again presents a major problem for assessing the value of results obtained in this assay. The criteria used to select the exposure levels were not presented. Generally, in such a study, concentrations are selected by first determining a dose which can cause profound cytotoxic effects as judged by trypan blue exclusion, LDH release, cell detachment, or altered morphology. Normally, test doses that are significant fractions of the cytotoxic dose would not be selected for testing. However, there is no indication that any cytotoxicity measurements were made in these studies.

A second criticism of the study results from the method used to score exposed silver grains over hepatocytes. In the standard protocol the authors state: "For each cell the number of grains over the nucleus and the highest number of grains in an equivalent adjacent area of cytoplasm were counted. The cytoplasmic count was subtracted from the nuclear count to give a net grain value for each cell." This method of selecting the highest number of grains in an equivalent adjacent area has been commonly used in the past. New guidelines from the American Society for Testing and Materials, agreed upon by experts in the field, state that an average number of grains from several equivalent adjacent areas of cytoplasm should be used to quantify the background. This change could significantly alter the data and conclusions in a study such as this one in which the results at the high dose are equivocal.

Finally, data interpretation presents an additional problem for evaluating the merits of this study. Both the rat and mouse *in vitro* studies were judged to be negative; however, the HRAC judges them as equivocal in result. For example, in the mouse study, 1.3% of the cells were in repair for the control, while 13.3% were in repair for the 500 ppm cultures, 10.0% for the 1000 ppm cultures, 14.0% for the 2000 ppm cultures, and 19.3% for the 4000 ppm cultures. Thus, there would appear to be an obvious dose response. The authors provide no statistical basis for calling these results negative. Clearly, this difference is much larger than that seen between the negative and positive controls

in the rat study: 8.5% and 10.6%, respectively. Therefore, if the difference for the mouse assay is not significant, then the controls for the rat assay are not valid.

Finally, in the discussion section the investigators state "It is however clear that methylene chloride is exerting a low level non-specific effect on both nuclear and cytoplasmic grain counts." The authors' explanation of a low level non-specific effect was not clear. Furthermore, the authors speculated that this effect may represent "an early indication of elevated levels of replicative (scheduled) DNA synthesis." It is not clear how the authors may have arrived at this conclusion, since DNA replication still takes place in the nucleus.

5.1.3. Covalent Binding to DNA in vivo

This study (CEFIC, 1986c) attempts to determine whether the carcinogenic action of DCM may be mediated through its ability to bind covalently to DNA and thereby lead to mutations. Fischer 344 rats and B6C3F1 mice were exposed to 4000 ppm ^{14}C -DCM for 3 hours. DNA was isolated from lung and liver tissues 6, 12, and 24 hours after the start of exposure, then hydrolyzed and analyzed by high performance liquid chromatography. As a control, an additional group of rats and mice were injected with ^{14}C -formate to determine if radioactivity associated with DNA originated from incorporation of radiolabeled carbon from the free carbon pool. The rationale for this study is based on the observations that DCM, which can be metabolized in several ways, is believed to yield reactive intermediates capable of alkylating

DNA and thus leading to mutations, but DCM can also generate formate as an intermediate which can enter the free carbon pool. Since this pool is used in the normal biosynthesis of nucleotide bases, radioactivity associated with DNA after exposure to labeled DCM may be a normal component of DNA and not an alkylated product. Based on the results of this study the authors indeed conclude that all the radioactivity associated with DNA after exposure to ^{14}C -DCM results from incorporation of formate into nucleotide bases and not from DNA covalent binding. The authors further conclude that based on these results, DCM is not genotoxic and is another example of an "epigenetic carcinogen."

This study can be criticized on several grounds. First, it is important to note that chemicals can cause mutations in DNA without alkylation. For example, intercalating agents can cause frameshift mutations without covalent binding, and some metals have been shown to cause mutations in DNA by interfering with the fidelity of DNA polymerase. Therefore, the assertion, that because DCM does not bind to DNA it is not genotoxic, is invalid. Second, the authors again performed the studies only in male animals without explanation.

The major weaknesses of this study, however, appear to be in design and interpretation. First, it is not clear why the investigators chose to dilute ^{14}C -DCM with unlabeled compound to produce a lower specific activity exposure environment. Clearly, the higher the specific activity, the greater the probability of detecting a minor DNA adduct. Second, the authors present an

elaborate argument to support their conviction that the radioactivity in the second fraction of the mouse liver chromatogram is really a protein contaminant containing radiolabeled amino acids which were also synthesized from the free carbon pool. To support this argument the authors point out that a similar peak (although relatively smaller) is also present in the chromatogram from livers of mice exposed to ^{14}C -formate and in the chromatogram of lungs from mice exposed to ^{14}C -DCM. However, the relative size of this peak in the lung chromatogram is greatly diminished. In order to reconcile this apparent discrepancy, the authors present the following highly elaborate but specious argument:

The fact that similar contamination by radioactive protein is not seen in mouse lung DNA may be a reflection on the known specificity of methylene chloride for a single cell type in the lung, the Clara cell (CTL/P/1432). In the liver, even though there are small differences in the distribution of metabolizing enzymes across the hepatic parenchyma most hepatocytes will metabolize methylene chloride to a similar extent. Consequently protein and DNA in each hepatocyte will have been exposed to a similar concentration of methylene chloride metabolites and the macromolecules isolated from the whole organ will be reasonably representative of each single cell within the liver. In contrast to the liver, the specific damage to mouse lung Clara cell suggests that metabolism occurs largely in a single cell type which is known to contain the highest concentration of cytochrome P-450 enzymes in the lung (Boyd, 1977). Because the Clara cell constitutes approximately 5% of the total cell types in mouse lung, specificity for this cell would result in a 20-fold dilution in the specific activity of protein isolated from the whole organ. In contrast, ^{14}C -formate incorporation into DNA will presumably occur in all cell types of the lung dependent only on their rates of DNA synthesis. The effect therefore of protein contamination of DNA from the lung will be markedly less than for hepatic DNA.

Such reasoning fails to consider that if only the Clara cell metabolized DCM to formate, permitting ^{14}C into the free carbon pool to be used in protein synthesis, the radioactivity in the DNA will also only be derived from Clara cells. Thus, the ratio of radiolabeled DNA to protein should remain the same, although the specific activity of both macromolecules should be lower in lung. The specific activity of DNA from the two tissues is essentially the same, while the specific activity for lung proteins is not provided in the report. In any case, this issue could have been resolved easily and directly by simply injecting one of the mice with a tritiated amino acid and then analyzing for both labels in the chromatographic fractions. If the authors' hypothesis is correct, tritium should only be detected in fraction 2.

Finally, the authors have ignored all of the labeled peaks in fractions 22 through 27 in the chromatogram of mouse liver DNA. Yet, these peaks could have coincided with alkylated bases. Indeed, the use of a higher specific activity DCM would probably have enhanced these peaks. The sensitivity of the assay poses a further problem. In examining the chromatograms of the DNA hydrolyzates, it is clear that there is radioactivity associated with all nucleotide bases except cytosine. This is as expected since the free carbon pool is used by the cell in the synthesis of purine rings but not in the synthesis of pyrimidine ring structures. However, radiolabel is expected to be associated with thymidine since the 5-methyl group is derived from the free

carbon pool. Concern is raised because there is no peak associated with 5-methyl cytosine. This modified base is created after cytosine is incorporated into DNA and should comprise approximately 5% of total cytosine; the source of this methyl group is the free carbon pool. One can conclude, therefore, that this assay is incapable of detecting a single species of alkylated nucleotide even when that species comprises 5% of the total amount of that base.

5.1.4. Summary of Phase I Tests

The results of mutagenicity testing of DCM are mixed. DCM clearly is a bacterial mutagen giving positive responses in the Ames assay (Barber et al., 1980; Green, 1983; Jongen et al., 1982). Both positive and negative responses have been reported in the yeast Saccharomyces cerevisiae (Callen et al., 1980; Simmon et al., 1977) in *Drosophila* (Abrahamson and Valencia, 1980; Gocke et al., 1981), as well as for the induction of sister chromatid exchange in cultured hamster cells (Jongen et al., 1982; Thilagar and Kumaroo, 1983). Highly significant levels of chromosomal aberrations were induced in CHO cells in vitro (Thilagar and Kumaroo, 1983) but were not detected in vivo (Dow Chemical Company, 1980); the doses tested in this latter study may not have been sufficiently high. The weight of evidence suggests that DCM is mutagenic, although it is perhaps a weak mutagen. The results suggest that DCM, like other mutagenic carcinogens, initiates cancer through genetic alterations. In addition, other health effects associated with exposure to

mutagens may also be of concern, e.g., heritable (germ line) mutations, teratogenicity, and reproductive effects. The data presented by the ICI-UK provide nothing to alter this conclusion.

5.2. PHASE II TESTS

In Phase II of its investigation into the carcinogenic mechanism of DCM, CEFIC (1986d, g) tested the effects of the compound on rats and mice in two additional assays: the mouse micronucleus test and induction of S-phase hepatocytes.

5.2.1. Mouse Micronucleus Test

DCM was tested (CEFIC, 1986d) in the mouse micronucleus test using both male and female C57BL/6J/Alpk mice. Preliminary studies were performed to determine the approximate MLD/7 (mean lethal dose after 7 days). The micronucleus test was performed using doses of 4000 mg/kg, 2500 mg/kg, and 1250 mg/kg, which are equivalent to 80%, 50%, and 25% of the MLD/7, respectively. Ten mice were included in each dose group, along with positive (cyclophosphamide) and solvent (corn oil) controls. Animals were sacrificed and bone marrow smears prepared at 24, 36, 48, and 72 hours after dosing. The frequencies of micronuclei per polychromatic erythrocyte (PCE) were within the control range for all dose levels and all time points. The percentage of PCEs was decreased for the 24-hour time points at the two highest doses, although statistical significance was achieved only at the 2500 mg/kg dose.

The authors concluded that DCM is not clastogenic in the mouse micronucleus test. The HRAC believes a more accurate

conclusion would be that DCM was not positive in the mouse bone marrow micronucleus test (under the conditions of the assay). This is because the micronucleus test does not detect all clastogenic activity, but only acentric fragments and some spindle effects. Also, although bone marrow is generally used for micronucleus assays, bone marrow is not a target for DCM, and reactive metabolites may not reach it in sufficient quantity to produce a detectable effect.

5.2.2. Induction of S-phase Hepatocytes

DCM was tested (CEFIC, 1986g) for its ability to induce DNA synthesis in liver cells of B6C3F1 mice. The induction of S-phase hepatocytes was measured after either one or two inhalation exposures to DCM for 2 hours. After exposure the animals were injected with [methyl-³H] thymidine using one of three radiolabeling regimens, and DNA synthesis was quantified using autoradiography at 24 and 48 hours after exposure. Sodium phenobarbitone was used as the positive control. The use of two of the three experimental protocols revealed small but statistically significant increases in the numbers of S-phase cells.

The results of these studies are difficult to interpret for a number of reasons. The induction of S-phase hepatocytes is not a commonly used assay, and the results, either positive, negative, or as in this case equivocal, are of unknown significance. The authors state:

The carcinogens [carbon tetrachloride, trichlorethylene, and polybrominated biphenyls] do, however, induce liver

cell turnover in vivo, as monitored by increased DNA scheduled synthesis. It has therefore been suggested that such as increased cell turnover in the liver may be mechanistically involved in the hepatocarcinogenicity to the mouse of compounds found to be non-genotoxic in vivo. Mirsalis et al. (1985) have suggested that the apparent correlation between induced cell proliferation in the liver and hepatocarcinogenicity indicates that any compound that induces increased cell turnover in the B6C3F1 mouse liver may produce liver tumors in a two year bioassay.

Based on the explanation in the section above and the use of phenobarbital as the positive control, the S-phase induction assay appears to be a test for potent promoters in a system that is genetically "initiated." It is clear, however, that simply inducing liver cell turnover alone cannot be the entire action of the hepatocarcinogens described above, since a partial hepatectomy, a very efficient inducer of hepatocyte DNA synthesis is not, in and of itself, carcinogenic (European Chemical Industry Ecology and Toxicology Center, 1982). Therefore, such "non-genotoxic hepatocarcinogens" must have other actions in addition to the induction of cell replication. Furthermore, since the authors claim that the small increases seen in this study may have no biological significance, one may conclude that DCM does not fall into this class of carcinogens, and therefore the low levels of genotoxicity seen in in vitro assays may be responsible for its carcinogenic activity.

The equivocal results seen in this study may be based on the doses of DCM that were selected for study: 4000 ppm for 2 hours for a single or two inhalation exposures. This was the same dose used in the NTP bioassay, but, in the latter, mice were exposed

to this same level for 6 hours a day, 5 days a week, for a total of 2 years. The exposures used in this acute study may have been too low or of insufficient duration to maximally induce DNA synthesis.

Finally, the liver was not the only target organ of DCM-induced tumors. Lung tumors were induced as well.

5.2.3. Summary of Phase II Tests

The mouse bone marrow micronucleus test was conducted at adequate exposure levels (CEFIC, 1986d). The negative results may have been due to the fact that bone marrow is not a target organ for DCM. The induction of S-phase, even if it were clear-cut, could not be interpreted at this time in a way that would help to elucidate the mechanism of DCM carcinogenesis.

There is, at present, no way to discriminate between nongenotoxic and weakly genotoxic activity for DCM.

6. EPIDEMIOLOGY: RECENT KODAK STUDY

6.1. INTRODUCTION

Since the Health Assessment Document (HAD) for DCM was published, Friedlander et al. have updated their study of Eastman-Kodak employees, increasing by some 262 men the size of the original cohort and presenting some detailed dose-response analyses. An early report dated July 2, 1985, had been submitted to the EPA; subsequently, the authors presented an updated analysis at the Winter Toxicology Forum in Washington, D.C., on February 18, 1986, and later at a meeting with scientists from the EPA, FDA, and CPSC. In June 1986, this analysis was submitted to EPA under the authorship of Hearne et al. (1986) as a prepublication copy. This paper has been published recently with only minor changes (Hearne et al., 1987). Two main themes are emphasized in these recent updates: first, that DCM is safe for humans at the occupational exposure levels; and second, that EPA's upper-limit incremental cancer risk estimates based on animal studies, significantly overpredict the cancer experience of the Kodak employees, and therefore should be lowered.

Other actions are also of note. The series of Kodak studies through July 2, 1985, were sent to Professor Genevieve Matanowski for review by EPA's Office of Toxic Substances; she submitted her report on October 15, 1985. This review was in turn responded to by Friedlander et al. on April 15, 1986. Matanowski responded to the Friedlander et al. response, but was, at the same time, part

of a team reviewing the Hearne et al. (1986) paper (Batelle, 1986). Matanowski's review dealt with qualitative rather than quantitative issues. Briefly, she noted some methodological problems which preclude her from agreeing with the authors' conclusions regarding the safety of DCM. One of her major criticisms was that the cohort actually was a survivorship cohort, with the associated bias being one of selection of persons remaining because of tolerance to the exposure conditions. She suggested that this bias must be removed before a meaningful analysis could be done. Matanowski also found problems with the control populations. Comparison of the Kodak death rates with the New York State death rates raised the "healthy worker effect" issue. While comparisons with the total Kodak hourly workers adjusted for the healthy worker effect, shortcomings of such a comparison were described. Matanowski did not, however, suggest a quantitative estimate of the bias involved in these SMR comparisons; as a result, no adjustment could be made.

The following sections present the HRAC review and analysis of the most recent Kodak study (Hearne et al., 1987). Section 6.1. presents a critical review of the methodology and results of the study; section 6.2. uses the data to calculate a quantitative cancer risk estimate, and compares this estimate with others that have been calculated both from human and animal studies; section 6.3. discusses the significance of the pancreatic cancer response; and section 6.4. summarizes the

results.

6.2. HRAC REVIEW OF THE STUDY BY HEARNE ET AL. (1987)

Hearne et al. (1987) completed a historic prospective study on all 1013 male employees of the film casting division of Eastman-Kodak in Rochester, NY (Kodak-Rochester), who were employed at least one year, of which some portion of that employment had to overlap into the period from January 1, 1964 through December 31, 1970. The cohort was followed through December 31, 1984, and vital status ascertainment was 99.1% complete. Causes of death in this cohort were contrasted with those expected based on rates of two referent comparison groups: (1) the general male population of upstate New York (excluding New York City) from 1965 to 1980, and (2) an occupational population of greater than 40,000 male employees of Kodak-Rochester (excluding this plant) from 1964 to 1985.

DCM has been used since 1944 in this plant as a solvent in the manufacture of cellulose triacetate film base. This process is accomplished in large hooded "casting machines" within which heated air is circulated and drawn off into a solvent recovery facility. Employees must enter the casting machines from time to time (up to six entries per day) to make manual adjustments and do maintenance. Samples from personnel industrial hygiene monitors and surrounding air indicated that concentrations of DCM range from 5000 to 10,000 ppm within the casting machines and between 30 to 100 ppm outside the casting machines but within the workroom. Air-supplied respirators were provided for use

whenever entry into the casting machines became necessary, but they were not always used.

The measurement of exposure appears to have been quite well-done. Since 1945 more than 1200 samples of area air and from personnel monitors had been collected at several locations in the vicinity of the casting machines, usually in the breathing zone of the employees. Additionally, to assist in operations, thousands of samples were taken by engineering personnel at critical points within the machines.

The characterization of DCM exposure also appears to have been done thoroughly. Regression analysis by the authors failed to reveal any significant trend in seasonally adjusted DCM concentrations within the production room between 1953 and 1985. An estimate of lifetime exposure for each employee was then derived by summing the products of the duration in months spent in each of these jobs by the average exposure level in ppm (normalized to an 8-hour time-weighted average) in each of those jobs. Three career exposure categories were defined: under 350 ppm-years, 350 to 749 ppm-years, and 750 ppm-years or greater. The median latency in each of these categories were 17, 31, and 37 years, respectively.

A priori hypotheses, based on the results of an NTP bioassay (1986) and in vivo studies of the metabolism of DCM, led the authors to consider malignancy of the lung and liver as well as ischemic heart disease as possibly high risk causes of death.

The authors found a statistically significant deficit of

deaths from all causes combined compared to the death rates of males living in upstate New York or employed at Kodak-Rochester [176 observed versus 253.2 expected (upstate New York) or 205.8 expected (Kodak-Rochester)]. With respect to total malignant neoplasms, a nonsignificant deficit of deaths was noted [41 observed versus 59.3 expected (upstate New York) or 52.7 expected (Kodak-Rochester)]. Deficits, albeit nonsignificant, were also seen for respiratory cancer [14 observed versus 21.0 expected (upstate New York) or 16.6 expected (Kodak-Rochester)] as well as liver cancer [0 observed versus 0.8 expected (upstate New York) or 0.5 expected (Kodak-Rochester)]. A statistically significant deficit of colon-rectal cancer is apparent [2 observed versus 9.8 expected (upstate New York)], as well as a nonsignificant excess risk of pancreatic cancer [8 observed versus 3.2 expected (upstate New York) or 3.1 expected (Kodak-Rochester)] attributed by the authors to possible misdiagnoses or incorrect coding of underlying cause of death.

This study appears to be well-conducted in all respects except one. There is a distinct possibility that the cohort selection process may have been faulty. Approximately 75% of the cohort began work prior to January 1, 1964, but after 1944. A total of 48% of the cohort actually began employment prior to 1954. Anyone who terminated his employment for whatever reason prior to January 1, 1964, was not included in the cohort. This cohort selection criterion has the potential for providing a study cohort that consists mainly of "survivors" (non-

susceptibles, exceptionally careful health-conscious persons, or persons who just did not succumb to a work-related illness). This phenomenon is different from the healthy worker effect which was present when observed deaths were contrasted with upstate New York death rates but not when contrasted with deaths rates generated from Kodak-Rochester employees. However, even in the latter case severe deficits still persist with some exceptions, i.e., lung cancer. These results are suspicious unless the survivorship question is considered as a contributing factor. That this survivorship effect is present is supported by the analysis of respiratory cancer by latency and exposure. There appears to be a nonsignificant elevated risk of respiratory cancer in the lowest exposure and latent categories where one would expect that the experience of the more recent hires would predominate. This is followed by a decrease in risk as exposure or period of follow-up increases. A larger proportion of the experience of the pre-1964 hires tends to predominate in the higher dose and latent categories. This phenomenon has also been seen in other studies but most notably in the Fox and Collier (1978) studies of vinyl chloride workers.

One possible solution to this problem would be to either include those employees terminated prior to 1964 in the cohort and blend their mortality experience in with the present cohort. Alternatively, the analysis could exclude all employees who began prior to January 1, 1964, and examine only the mortality experience of those employed on or after January 1, 1964. The

main deficiency with the latter solution is that very few person-years would accumulate in the higher latency and dose categories thus leading to a tremendous drop in the power to detect an increased risk if one were present. If these suggestions are incorporated into the study design and the results of the reanalysis are nonsignificant, then it seems likely that this study could be used to determine an upper-bound risk estimate for cancer. In the latter solution, however, where the power is dramatically reduced, the results could not be assumed to imply that exposure to DCM does not increase one's risk of cancer.

6.3. APPLICATION OF EPIDEMIOLOGY TO QUANTITATIVE CANCER RISK ASSESSMENT AND COMPARISON WITH ANIMAL-BASED ESTIMATES

This section discusses several previous quantitative cancer risk assessments for DCM. In the next section a comparison will be made of the upper-limit incremental unit cancer risk estimate for DCM predicted from the NTP mouse inhalation lifetime bioassay (1985, 1986) with that predicted from the Hearne et al. (1987) study of Kodak employees.

6.3.1. Previous Quantitative Cancer Risk Assessments for DCM

The Carcinogen Assessment Group (CAG) has previously reviewed both the animal studies (NTP, 1985) and epidemiologic studies (up to and including Hearne and Friedlander, 1981) in the HAD for DCM and Addendum (U.S. EPA, 1985a, b). Based on the studies, CAG calculated an upper-limit incremental unit risk of $q_1^* = 1.4 \times 10^{-2} \text{ (ppm)}^{-1}$ for inhalation based on lung and liver

tumors in mice. The CAG has also calculated 95% upper-limits of expected deaths based on the early epidemiology study and concluded that the "Friedlander et al. study does not have the power to rule out an overall cancer risk, or . . . a lung cancer risk, that is predicted using the upper-bound slope derived from the NTP study." Because the Hearne and Friedlander (1981) update was considered a negative response for cancer, if the upper-limit risk estimate based on the epidemiology study had been less than that based on the animal data, a human data estimate would have been used.

Allen et al. (1986) prepared a risk assessment of the Hearne and Friedlander 1981 update of their original 1978 study as part of an overall effort to correlate risk assessments based on animal and human data for the CAG. Allen et al. based their assessment on a relative risk model and 24 total malignant neoplasms observed in the cohort update of the 1981 paper versus 28.64 expected. Only average exposure data for the entire cohort were available. The results of this analysis are presented in Table 8. Also included in Table 8 are estimates which Allen et al. calculated based on a study of Dow Chemical Company employees. This upper-limit estimate, 1.6×10^{-2} (ppm)⁻¹, is also consistent with that based on total cancer deaths in the Kodak study, but Allen et al. used a methodology for uncertainty which tends to inflate the upper-limit estimates versus the EPA methodology.

With respect to the Hearne et al. (1987) update, the U.S.

TABLE 8. COMPARISON OF PREVIOUS INCREMENTAL CANCER RISK ESTIMATES FOR DCM BASED ON PANCREATIC, LUNG, AND TOTAL CANCER DEATH RESPONSE IN THE KODAK AND DOW STUDIES

| Study | Risk model used | Cancer type | Lifetime incremental cancer risk per 1 ppm continuous exposure | | | |
|-------|--------------------|--------------------|--|-----------------------------|----------------------|--|
| | | | Lower limit | Maximum likelihood estimate | 95% upper limit | |
| Kodak | Additive | Pancreasa | 0 | 6.7×10^{-3} | 3.0×10^{-3} | |
| | Relative | Pancreasa | 0 | 4.7×10^{-3} | 7.9×10^{-3} | |
| | | Lunga | 0 | 0 | 3.5×10^{-3} | |
| Dow | Total ^b | Total ^b | 0 | 0 | 1.0×10^{-2} | |
| | | Total ^b | 0 | 0 | 1.6×10^{-2} | |

^aState of California, 1986.

^bAllen et al., 1986.

Consumer Product Safety Commission (CPSC) has examined the authors' claim that upper-limit estimates based on animal data are too high (Cohn and Rock, 1986). The reanalysis by CPSC found that extra cases predicted for the Kodak employees from animal data were between 2.2 and 8.7, estimates which bordered the 4.8 excess of pancreatic cancers observed in Kodak employees in the most recent update. Another agency, the California Department of Health Services, prepared a report (1986) with an analysis of more extensive dose-response data obtained personally from the authors of the Kodak study. In that analysis upper-limit unit risk estimates were developed based on both lung and pancreatic cancers in the Kodak workers. The upper-limit estimates based on pancreatic cancers are within a factor of plus or minus 2 of the EPA's upper-limit estimates based on the NTP female mouse bioassay, which is $q_1^* = 1.4 \times 10^{-2} \text{ (ppm)}^{-1}$. These estimates are presented in Table 8.

One other analysis of the NTP mouse data, an internal Food and Drug Administration (FDA) memorandum dated May 4, 1985, estimated a unit risk value roughly 27.6 times less than EPA's. There are also other analyses using epidemiologic data, including the Dow study on DCM workers (U.S. EPA, 1985a). The bulk of these analyses, however, discuss the epidemiologic studies in terms of statistical power concepts, i.e., the probability of detecting an increase in cancers if one is actually associated with DCM exposure. While EPA has dealt with this concept in a previous document on DCM (U.S. EPA, 1985a) and other chemicals,

the focus here will be on the analysis of actual risk estimation based on the Kodak employee cohort, which is presented in the following section.

6.3.2. Quantitative Risk Estimation Based on the Study of Kodak Employees

The following analysis provides maximum likelihood and 95% upper-limit estimates of incremental cancer risk based on the cancer death response in the lung and pancreas in the most recent update of the Kodak study (Hearne et al., 1987). Both additive and relative risk models are used. This type of analysis has been used previously with epidemiologic studies in several EPA documents; the HADs for nickel and cadmium are two recent examples. It also follows closely the analysis used by the State of California (1986), but the results differ--in some cases only slightly, in others quite a bit--because of an adjustment made for latency. A description of the models follows.

6.3.2.1. Excess or Additive Risk Model--This model follows the assumption that the excess cause-age-specific death rate due to DCM exposure, $h_1(t)$, is increased in an additive way by an amount proportional to the cumulative exposure up to that age. In mathematical terms, this is

$$h_1(t) = BX_t$$

where X_t is the cumulative exposure up to age t , and B is the proportional increase. The total cause-age-specific rate $h(t)$ is

then additive to the background cause-specific rate $h_0(t)$ as follows:

$$h(t) = h_0(t) + h_1(t)$$

Under the assumptions of this model, the parameter B can be estimated by summing the expected rates to yield

$$E_j = E_{0j} + BX_jW_j$$

where E_j is the total number of expected cancer deaths in the observation period from the group exposed to cumulative exposure X_j . E_{0j} is the expected number of cancer deaths due to background causes; for the Kodak study, expected deaths based on both total Kodak employees and New York State cancer death rates are available. The total Kodak employee death rates were used since they eliminated the "healthy worker effect," although, as discussed in Section 6.2., this control group may not be entirely suitable. W_j is the number of person-years of observation for the j th exposure group, and the parameter B represents the slope of the dose-response model. To estimate B, the observed number of cause-specific deaths, O_j , is substituted for E_j . O_j is assumed to be distributed as a Poisson random variable. The parameter estimate, b , can be tested for being significantly greater than zero. A statistically significant result is evidence of an additional cancer effect due to cumulative DCM

exposure.

Under the above assumptions, the solution by maximum likelihood proceeds as follows: the likelihood equation is

$$L = \prod_{j=1}^N [\exp-(E_{0j} + BX_jW_j)] [E_{0j} + BX_jW_j]^{O_j} / O_j!$$

where N = the number of separate exposure groups. The maximum likelihood estimate (MLE) of the parameter B is obtained by solving the equation

$$\frac{d \ln L}{dB} = \sum_{j=1}^N [-X_jW_j + O_jX_jW_j] / (E_{0j} + BX_jW_j) = 0$$

for B.

The asymptotic variance for the parameter estimate b is

$$\left[\sum_{j=1}^N X_j^2 W_j / (E_{0j} + bX_jW_j) \right]^{-1}$$

where b is the MLE. This variance can then be used to obtain approximate 95% upper and lower bounds for b. Lifetime incremental cancer risk estimates for 1 ppm continuous exposure are estimated by multiplying b by 70 if X is in units of lifetime continuous exposure.

6.3.2.2. Multiplicative or Relative Risk Model--This model follows the assumption that the background cause-age-specific rate at any age t is increased in a multiplicative way by an

amount proportional to the cumulative dose up to that age. In mathematical terms this is

$$h(t) = h_0(t)(1 + BX_t)$$

As above, summing over the observed and expected experience yields, for each exposure group,

$$E_j/E_{0j} = 1 + BX_j$$

Again, to estimate B, the observed number of cause-specific deaths, O_j , assumed to be a Poisson random variable, is substituted for E_j . Following the same procedure as above, the MLE, b , is the solution to

$$\frac{d \ln L}{dB} = \sum_{j=1}^N [-E_{0j}X_j + (O_jX_j)/(1 + BX_j)] = 0$$

with asymptotic variance

$$\left[\sum_{j=1}^N (E_{0j}X_j^2)/(1 + bX_j) \right]^{-1}$$

Lifetime incremental risk estimates per ppm under this model are obtained by multiplying b by the background lifetime cause-specific risk of death, P_0 . The values P_0 are derived using life table methods and 1980 U.S. death rates. For lung and pancreas cancers these are 0.037 and 0.008, respectively.

6.3.2.3. Data--The data for the above models, presented in Tables 9 and 10, are derived from information supplied to the State of California by Dr. Friedlander of Kodak and are reproduced from the State of California's criteria document. Table 9 presents the observed and expected deaths from lung cancer and Table 10 presents the pancreatic cancer death experience by separate age and exposure categories. The three career cumulative exposure categories (<350, 350-749, and 750+ ppm-years) are the same as those presented in the Hearne et al. (1987) update and are comprised of 350, 353, and 310 employees, with mean exposure levels of 16, 22, and 42 ppm (8-hour time-weighted average) respectively. Mean duration of exposure (13, 26, and 29 years) and median latency (17, 31, and 37 years) also increased for the higher level exposure categories. The mean cumulative exposures were 153, 531, and 1212 ppm-years, respectively. Lifetime continuous exposure (LCE), as presented in Tables 9 and 10, were calculated as follows:

$$LCE = X_1 = 16 \text{ ppm} \times 8/24 \times 240/365 \times 13/70 = 0.65 \text{ ppm}$$

For the two higher exposure categories, substitution of mean exposure levels 22 and 42 ppm, and mean duration of 26 and 29 years, respectively, leads to LCEs of 1.79 ppm and 3.81 ppm.

The State of California analysis used all age and exposure groups for every analysis. Here all experience prior to age 45 is eliminated, on the assumption of a 20-year latency and the

TABLE 9. PERSON-YEARS OF OBSERVATION AND OBSERVED AND EXPECTED DEATHS
FROM LUNG CANCER FOR KODAK EMPLOYEES EXPOSED TO DCM WITH FOLLOW-UP THROUGH 1984

| Cohort | Age group | | | | | | Total |
|--|-----------|--------|--------|--------|--------|--------|-------|
| | 15-25 | 25-34 | 35-44 | 45-54 | 55-64 | 65-74 | |
| <350 ppm-years ($ICF=X_j=0.65$): ^a | | | | | | | |
| Person-years (W_j) | 1109.9 | 3381.7 | 2808.3 | 1047.4 | 612.8 | 306.2 | 88.2 |
| Lung cancer deaths: | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 0 | 3 | 1 | 0 |
| Expected (E_{0j}) | 0.020 | 0.000 | 0.269 | 0.599 | 0.752 | 0.999 | 0.416 |
| 4 | | | | | | | |
| 350-749 ppm-years ($ICF=X_j=1.79$): | | | | | | | |
| Person-years (W_j) | 0 | 135 | 1737.1 | 1986.9 | 1334.0 | 683.8 | 132.0 |
| Lung cancer deaths: | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| Expected (E_{0j}) | 0.000 | 0.000 | 0.166 | 1.136 | 1.635 | 2.231 | 0.622 |
| 5 | | | | | | | |
| 750+ ppm-years ($ICF=X_j=3.81$): | | | | | | | |
| Person-years (W_j) | 0 | 0 | 165.8 | 1112.3 | 1643.5 | 1005.2 | 174.8 |
| Lung cancer deaths: | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 0 | 2 | 2 | 1 |
| Expected (E_{0j}) | 0.000 | 0.000 | 0.016 | 0.636 | 2.015 | 3.280 | 0.824 |
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$aICF = 16 \text{ ppm} \times 8/24 \times 240/365 \times 13/70 = 0.65 \text{ ppm}$.

SOURCE: State of California, 1986.

TABLE 10. PERSON-YEARS OF OBSERVATION AND OBSERVED AND EXPECTED DEATHS FROM PANCREATIC CANCER FOR KODAK EMPLOYEES EXPOSED TO DCM WITH FOLLOW-UP THROUGH 1984

| Cohort | Age group | | | | | | | | | | Total | |
|---|-----------|--------|--------|--------|--------|--------|-------|--|--|--|-------|-------|
| | 15-25 | 25-34 | 35-44 | 45-54 | 55-64 | 65-74 | 75+ | | | | | |
| <u><350 ppm-years</u> (LCE= $X_j=0.65$): | | | | | | | | | | | | |
| Person-years (W_j) | 1109.9 | 3381.7 | 2808.3 | 1047.4 | 612.8 | 306.2 | 88.2 | | | | | |
| Pancreatic cancer deaths: | | | | | | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 0 | 1 | 0 | 1 | | | | | 2 |
| Expected (E_{0j}) | 0.000 | 0.030 | 0.134 | 0.163 | 0.226 | 0.107 | 0.058 | | | | | 0.718 |
| <u>350-749 ppm-years</u> (LCE= $X_j=1.79$): | | | | | | | | | | | | |
| Person-years (W_j) | 0 | 135 | 1737.1 | 1986.9 | 1334.0 | 683.8 | 132.0 | | | | | |
| Pancreatic cancer deaths: | | | | | | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 1 | 0 | 1 | 0 | | | | | 2 |
| Expected (E_{0j}) | 0.000 | 0.001 | 0.083 | 0.310 | 0.493 | 0.239 | 0.087 | | | | | 1.213 |
| <u>750+ ppm-years</u> (LCE= $X_j=3.18$): | | | | | | | | | | | | |
| Person-years (W_j) | 0 | 0 | 165.8 | 1112.3 | 1643.5 | 1005.2 | 174.8 | | | | | |
| Pancreatic cancer deaths: | | | | | | | | | | | | |
| Observed (O_j) | 0 | 0 | 0 | 0 | 1 | 3 | 0 | | | | | 4 |
| Expected (E_{0j}) | 0.000 | 0.000 | 0.008 | 0.174 | 0.607 | 0.351 | 0.116 | | | | | 1.256 |

SOURCE: State of California, 1986.

observation that only 1110 person-years of observation occurred before the age of 25. This implies that very few men started work before the age of 25, so that all experience up to age 45 would be within a 20-year latency period. Overall, there were 29,364.4 person-years of experience among the 1013 men for an average observation (or latency) of 29 years per man. Removing the experience before age 45 takes away 9337 or 31.8% of the person-years. However, there were no observed deaths and less than one expected death for lung or pancreatic cancer in these age groups. By comparing Tables 9 and 10, one can note that both the person-years and the LCEs are the same in corresponding groups, and that for any age group (column), the ratio of expected deaths from pancreatic cancer to expected deaths from lung cancer is constant.

6.3.2.4. Results--The results of the analyses, presented in Table 11, compare MLE and 95% lower- and upper-limit estimates based on animal (both administered and metabolized dose) and human cancer experience. As can be seen, estimates based on the human pancreatic cancer deaths are very close to those of the female mouse based on administered dose. The MLE estimates, 1.2×10^{-2} (ppm)⁻¹ and 5.0×10^{-3} (ppm)⁻¹, based on the additive and multiplicative models, respectively, are slightly higher than the MLE of 3.2×10^{-3} (ppm)⁻¹ based on combined lung and liver tumors in the mouse. The 95% upper-limit estimates based on the Kodak experience bound the upper-limit estimate of 1.4×10^{-2} (ppm)⁻¹ based on lung and liver tumors in the mouse.

Table 11 also compares the results of the Kodak employees' analyses with estimates based on the metabolized dose in animals. It can readily be seen that the upper-limit risks predicted from human lung cancers by either the additive or multiplicative model are very close to those predicted using metabolized dose from the animal data. Further, it can be seen that the upper-limit risk predicted from human pancreatic cancer deaths is greater than any of the risks predicted from animal data using metabolized dose.

Also included in Table 11 are tests of significance of the estimates based on the Kodak employees' experience. As can be seen, the p-values based on pancreatic cancer are borderline significant ($p = 0.05$) by the asymptotic normal test. The p-values based on the likelihood ratio test are $p = 0.02$ for the relative risk model and $p = 0.04$ for the additive risk model. For lung cancer, none of the p-values are statistically significant, and in fact, the MLE dose-response estimates are actually negative as might be expected from the data.

6.4. DISCUSSION

The above analysis shows that the estimates of incremental unit cancer risk based on the NTP female mouse inhalation study are about the same as those calculated from an analysis based on pancreas cancer deaths in the Kodak employees. The larger question remaining, however, is whether the pancreas cancer response is a true response. Two factors that appear significant are the concurrent decrease in colon-rectum cancers and the appearance of most of these pancreatic cancers between the last

TABLE 11. COMPARISON OF INCREMENTAL CANCER RISK ESTIMATES FOR DCM BASED ON THE NTP FEMALE MOUSE LUNG RESPONSE WITH ESTIMATES BASED ON LUNG AND PANCREAS CANCER DEATH RESPONSE 1013 KODAK EMPLOYEES

| Species/ sex | Model used | Cancer type | Parameter estimates b | Asymptotic variance estimates | p-value ^a | Lifetime incremental cancer risk per 1 ppm continuous exposure | | |
|------------------|--------------------------|----------------------------------|-----------------------------|-------------------------------------|----------------------|---|---|--------------------------------------|
| | | | | | | Lower limit | Maximum likelihood estimate | 95% upper limit |
| Humans/ males | Additive | Pancreas | 1.74x10 ⁻⁴ | 1.17x10 ⁻⁸ | 0.054 | 0 | 1.2x10 ^{-2b} | 2.5x10 ⁻² |
| | | Lung | -1.78x10 ⁻⁴ | 1.40x10 ⁻⁸ | 0.067N | N | N | 1.1x10 ⁻³ |
| | Multiplicative | Pancreas | 0.623 | 0.138 | 0.048 | 0 | 5.0x10 ⁻³ | 9.9x10 ⁻³ |
| | | | P ₀ =0.008 | | | | | |
| Mice/ females | Linearized Multistage | Lung and liver combined | -0.061 | 6.7x10 ⁻³ | 0.228N | N | N | 2.7x10 ⁻³ |
| | | | P ₀ =0.037 | | | | | |
| ----- | | | | | | | | |
| | | | | | | | Based on Administered dose Metabolized dose | |
| | | | | | | 0 | 3.2x10 ⁻³ | 1.4x10 ⁻² |
| | | | | | | 0 | -- | (1.59 to 5.4 x10 ^{-3c}) |

^aBased on two-tailed asymptotic normal test of parameter.

^b b x 70.

^cSee Chapter 7.

N = negative estimate.

observation date, 1977, and the current one, 1984. The Batelle report (1986) suggests that in the earlier Friedlander et al. (1978) report there was an excess of stomach and gastrointestinal cancer deaths and that this excess might represent some misdiagnosed pancreatic cancer deaths. Regardless, Batelle suggests that the current standardized mortality ratio (SMR) of 250 for pancreatic cancer in a healthy population, an apparent rapid rise in the last 8 years of follow-up, plus the fact that no other disease shows such an effect, is, at least, cause for further investigation.

Hearne et al. (1987) argue the opposite. They suggest that when "a large number of non-hypothesized causes [are] evaluated, it is likely that [a significant result might happen] by chance alone." They point to "deficits of the same magnitude . . . for such sites as colon-rectum (2 vs. 9.8) and prostate and bladder (3 vs. 8.0)." They also suggest five other issues which, they claim, demonstrate the pancreatic cancer observation as a chance occurrence. These are: (1) no dose-related effect; (2) no evidence that DCM or its metabolites concentrate in the pancreas or produce any other toxic pancreatic response; (3) the histology for these cancers were adenocarcinomas, the most common pathologic diagnosis for this site; (4) potential concurrent exposure to suspected animal carcinogens 1,2-dichloroethane and 1,2-dichloropropane; and (5) each of the eight cases had one or more non-occupational risk factors [smoking (7), diabetes (2), and alcohol abuse (1)].

While both sides have merit, the HRAC believes that the above analysis, showing a statistically significant exposure-response trend for human pancreatic cancer of $0.01 < p < 0.05$, based on either the additive or multiplicative models, is suggestive of a real effect. Hearne et al. claim "no dose-related effect was observed (chi square trend = 0.02, p = 0.89)," but they do not display enough data for their numbers to be verified. From their data tables, it appears that their test was based on comparing SMRs for the three career-exposure categories. It is not clear how a chi square trend test can be successfully computed with these data. The HRAC believes that the above analysis, based on modeling, makes maximum use and weighting of the available data and, therefore, provides the appropriate trend test.

Examination of the literature provides only a suggestion that pancreatic cancer might be associated with the film-making process. Milham (1976) reported an excess of four to nine pancreatic cancer deaths and a risk ratio ≥ 2 among motion picture projectionists in Washington State between 1950 and 1971. However, the results were based on a highly unreliable proportionate mortality analysis, and the study design allowed for no additional analyses. Furthermore, there were no exposure measurements.

Finally, the argument of whether or not the pancreatic cancer response is a bona fide response has a bearing on the quantitative risk estimation. If the evidence for pancreatic

cancer in humans is considered limited and DCM is classified as a B1 carcinogen according to EPA's classification scheme, then the cancer risk estimate would be based on the MLE, 1.2×10^{-2} (ppm)⁻¹ or 5.0×10^{-3} (ppm)⁻¹ for the additive or multiplicative model, respectively. Both of these estimates are slightly lower than the upper-limit estimate based on the administered dose in the NTP study. However, they are both slightly higher than the upper-limit estimates based on the metabolized dose in the NTP study. If the pancreatic cancer response is considered possibly a chance occurrence and DCM receives a B2 classification, then the study would be used only to get the 95% upper-limit estimate on a negative-response study, and the risk estimates would be even higher. Thus, whether DCM is classified as a B1 or a B2 affects the quantitative risk estimates but in an inverted manner. This all, of course, assumes that the control population of all Kodak hourly employees is a proper comparison group.

6.5. SUMMARY

A quantitative risk extrapolation based on pancreatic cancer deaths in Eastman-Kodak employees exposed to an 8-hour time-weighted average of 30 to 125 ppm DCM yields 95% upper-limit estimates in the range of 9.9×10^{-3} to 2.5×10^{-2} (ppm)⁻¹. This range (Table 11) exceeds the proposed EPA upper-limit estimate derived from lung and liver tumors in the NTP female mouse inhalation study (based on metabolized dose). Furthermore, a test for exposure-response trend yields borderline statistical significance, additional evidence that the pancreatic cancer

response in the Kodak employees is exposure-related and not a chance occurrence. At this time, however, the HRAC believes that the evidence is not strong enough to determine if this response is bonafide and can merely conclude that the estimates based on animal cancers do not appear to overestimate the risk. Even if pancreatic cancer deaths are discounted, the 95% upper-limit estimates based on lung tumors from the epidemiologic analysis (Table 11) would be between 1.1×10^{-3} and 2.7×10^{-3} (ppm)⁻¹, which are comparable to the upper-limit estimates derived from the mouse metabolized dose data.

7. SCALING RISK ACROSS SPECIES USING DELIVERED DOSE

7.1. INTRODUCTION

Using experimental animal exposures to putative carcinogens as a means for estimating risk to humans relies on the general similarity of mammalian species in anatomy, physiology, and biochemistry. The biological processes that underlie carcinogenicity are not well understood, but it is reasonable to suppose that they will operate in a more or less similar manner in rodents and humans, at least in most cases. Although these processes may be supposed to be qualitatively similar, the rates at which they proceed will vary among species, leading to differences in the carcinogenic potency of a substance.

Much of the variation in the rates of the underlying processes will be the result of the pronounced difference in scale between humans and experimental animals. Humans are some 2000 times heavier than mice, they live about 35 times longer, and their physiological processes operate at a generally slower rate. The amount of a carcinogenic compound that engenders equal lifetime cancer risks in mice and humans clearly should depend on such differences. If one can properly take into account differences in scale, one can quantitatively predict human risks from observations on the doses that produce certain risks in animals.

Much controversy exists over the scaling factor that ought to be applied; different factors are preferred by different

federal regulatory agencies. FDA generally scales doses by body weight; doses are considered to be of equivalent lifetime carcinogenicity when the daily rate of dosing is equal in units of mg/kg. EPA and CPSC scale doses by body surface area. That is, doses are equivalent in units of $\text{mg}/\text{kg}^{2/3}/\text{day}$, since surface area is proportional to $(\text{body weight})^{2/3}$. (Both of these factors adjust for life span in that equivalent lifetime risks are based on daily exposure rates, even though humans are exposed for 35 times more days in a lifetime.)

The extrapolation of potencies of carcinogens using such scaling factors has some empirical support. For some chemicals, direct estimates of risk from defined exposures are available from epidemiologic data. Such estimates agree (at least generally) with projections based on scaling the potencies estimated from animal studies (National Academy of Sciences, 1975). Crump et al. (1985) and Allen et al. (1986) have shown for 23 "chemicals" (including industrial chemicals, drugs, and cigarette smoke) that the directly observed human cancer risks are well predicted from animal studies if the applied doses are scaled by some measure of body size and life span. Their data have insufficient resolution to clearly favor body weight or surface area as a scaling factor, however. Two points of caution should be noted. First, the chemicals in the comparison are ones producing rather high human risk, high enough to be estimated in epidemiologic studies. Thus, the extrapolation is from experimental animals at high bioassay doses to humans usually at

similarly high doses. Second, there is a good deal of variation among chemicals which reflects on the accuracy of the extrapolation; there is considerable room for improvement in prediction, especially in the area of "explaining" the outliers to the general pattern. Nonetheless, these studies are reassuring in their suggestion that current risk extrapolation processes are not completely ill-founded.

The question addressed in this chapter is how information on the internal target-organ doses of a putative carcinogen should affect the process of extrapolating risks observed in animals to humans. Extrapolation on the basis of applied or external dose can be criticized for failing to take into account species differences in metabolism and disposition, as well as for ignoring the changes in these factors from high doses to the low levels of exposure for which human risk estimates are usually desired. If information is available on internal doses in the tissues subject to carcinogenic induction, a dose measure much closer (in terms of the underlying causal processes) to the biological end point of concern can be examined. A discussion about the relationship of such data to the scaling process requires an examination of the scaling process as it is currently used on applied doses.

7.2. SCALING APPLIED DOSE TO EXTRAPOLATE RISK ACROSS SPECIES

The applied dose basis for risk extrapolation makes no explicit attempt to specify the underlying biological processes/factors that mediate the relationship between the dose

and the ultimate response. The same set of underlying processes is presumed to operate in experimental animals and in humans; the scaling factor is employed to account for the alteration in potency that results from the different values for factors affecting the underlying processes in larger, longer-lived humans compared to rodents. The scaling factor has to account for the overall scaling of the whole relationship of applied dose to response, even though that relationship comprises a large number of components. These components include the pharmacokinetic processes of absorption, distribution among the tissues, metabolism, and excretion, all of which will affect the degree to which the internal dose at the site of action remains proportional to the applied dose. Other components are those that affect the tissue's sensitivity to this internal dose, and include such factors as the cells' ability to scavenge free radicals, rates of DNA repair and cell turnover, the number of cells at risk, immunosurveillance, and others. The general empirical success of the scaling factors usually used by the federal regulatory agencies, discussed previously, argues that the combined effect of differences in all of the underlying factors is well estimated in many cases. However, no single factor can be identified as the key to explaining the interspecies differences in a carcinogen's potency, nor is the specific contribution of any one element to the overall scaling effect identifiable.

If pharmacokinetic data on humans and animals were available

on a number of the chemicals considered by Allen et al. (1986), one could compare the internal dose differences between species to the observed differences in the potency of carcinogens, yielding an estimate of the contribution of differences in pharmacokinetics to the scaling of potency across species. Such data are not currently available, however. Information on this question is being compiled by EPA with the hope of providing insights that will aid in interspecies extrapolation.

In order for pharmacokinetic models or data on internal doses to alter the risk extrapolation based on applied dose, one must replace the contribution of pharmacokinetics that has been assumed as part of the general scaling across species with the particular data for the compound at hand. In order to do so, one must make explicit the assumptions about the contribution of pharmacokinetics to interspecies scaling that one assumes in the applied dose extrapolation procedure. (Lacking data on the other elements contributing to sensitivity differences among species, one must assume that their contribution is the same as in the applied dose extrapolation.) If other pharmacodynamic factors are known or assumed based on appropriate evidence, the degree to which the data show that pharmacokinetic differences between humans and experimental animals do not follow the assumed pattern is the factor by which the risk extrapolation should be altered. Simply showing that species differences in pharmacokinetics exist does not help in deciding how to change human risk predictions, since the observed differences may be in accord with the general

scaling pattern between species that the applied dose scaling factor already encompasses.

The process of apportioning expected species differences in a carcinogen's potency between pharmacokinetic factors and other pharmacodynamic factors is difficult, and is discussed in the following section.

7.3. PHARMACODYNAMICS

Pharmacodynamics is "what the dose does to the body." Although pharmacodynamics can be defined to include pharmacokinetic considerations, it is defined for purposes of this discussion as including the biological processes that govern the target tissue's degree or probability of response to a given delivered dose, which may or may not be proportional to that delivered dose.

Our ignorance of pharmacodynamics is, if anything, greater than our ignorance of pharmacokinetics, especially when cancer is the end point. As in the case of pharmacokinetics, we ought to consider how pharmacodynamic differences from high to low doses and from species to species affect our ability to extrapolate risk from animal bioassays to human experience. Generally, if a carcinogen acts by enhancing or accelerating an ongoing biological process involved in the production of "background" tumors, we expect the carcinogenic potency of a delivered dose (at least at low levels) to be directly proportional to (i.e., to vary linearly with) that dose. However, a number of biological processes can lead to marked nonlinearity in the curve of

magnitude of effect against delivered dose. For example, the swamping of DNA repair at higher doses of an agent could cause the dose-effect curve to be convex (leading to lower than expected risks when extrapolated to low delivered doses). A carcinogen that must induce cytotoxicity to cause cancer could have a very convex dose-effect curve. On the other hand, if low doses of an agent fail to induce DNA repair enzymes, a somewhat concave dose-effect curve would result, and low dose risks may be underestimated. Although many possible nonlinear effects can be listed, their actual importance in real situations is currently unknown.

Clearly, different hypotheses about the mechanism of carcinogenic action profoundly affect the process of high- to low-dose extrapolation, even after pharmacokinetic nonlinearities are accounted for. One can make the assumption (if data are lacking) that within-species pharmacodynamics is linear; that is, the effect is directly proportional to the delivered dose. It is well to realize, however, that this assumption introduces uncertainty, and could be somewhat anti-conservative in some cases. On the other hand, risks from an epigenetic carcinogen that has little effect below a certain threshold will be overestimated.

The effect of pharmacodynamics on interspecies extrapolation of risk is a good deal more problematic. There are many factors that differ between rodents and humans that can be expected to influence the degree of toxic reaction to a given tissue-level

exposure. In the case of carcinogenicity, these include the slower rate of cell turnover in humans, the greater cellular defenses against free radicals and other highly reactive compounds, and possible differences in the efficiency of DNA repair. One major factor is that humans have many more cells in any given organ than do rodents, each of which will be at equally increased risk of carcinogenic transformation when exposed to a given tissue-level concentration of carcinogen. Since only one cell need be transformed to initiate a tumor, all else being equal, humans ought to be many times more sensitive to a given tissue concentration of a carcinogen than are rodents.

Clearly, all else is not equal. Interspecific factors that tend to increase human susceptibility to carcinogens are to a large degree balanced by other factors that tend to decrease susceptibility. The key question for interspecies extrapolation of carcinogenicity, which remains unanswered at the present time, is exactly where this balance is struck in humans vis-a-vis rodents. In other words, we do not yet know what to use for an "interspecies pharmacodynamic correction factor" to account for interspecific differences in the carcinogenic response of tissues to a given delivered dose.

The following section discusses the "surface area correction" as a means of scaling doses for interspecies extrapolation, in light of the uncertain contributions of pharmacodynamics and pharmacokinetics to the overall scaling of carcinogenic potency across species.

7.4. PHARMACOKINETICS, PHARMACODYNAMICS, AND THE SURFACE AREA CORRECTION

The surface area correction is the interspecies correction factor by which human applied doses (in mg/kg/day) are modified in order to be equivalent to animal doses (in the same units) under the EPA and CPSC assumption that doses are of equal risk when expressed in terms of $\text{mg/kg}^{2/3}/\text{day}$. Humans have a smaller surface area to volume ratio than do rodents, since surface area is approximately proportional to the $2/3$ power of body weight, while volume is approximately proportional to weight. Hence, an equal dose per $\text{kg}^{2/3}$ will be less per kg in humans. In extrapolating from mice weighing 0.0345 kg [which may be used for the weight of female mice in the NTP (1985) inhalation bioassay of DCM] to 70 kg humans, the human doses in mg/kg are divided by the cube root of the ratio of body weights, or a factor of 12.7.

In general terms, the overall interspecies correction factor for applied dose (F, which is equal to 12.7 in this case) must include the effects due both to interspecies pharmacokinetic differences (PK) and to interspecies pharmacodynamic differences (PD), both of which are usually unknown. One can represent this as a simple equation,

$$F = PK \times PD$$

where F is the multiplicative factor that relates human risk to mouse risk at the same applied dose in mg/kg/day, PK is the

factor that relates human delivered dose to mouse delivered dose at a given applied dose, and PD is the factor that relates human response at a given delivered dose to the mouse response at that same delivered dose. (To the extent that PK and PD display within-species nonlinearities, their values may change at different levels of applied dose; this phenomenon is ignored for the time being.)

When pharmacokinetic data are available, one must consider replacing the assumed value of PK with an empirical determination of this factor, PK* (where the "*" distinguishes an observed value from the a priori assumption). The new interspecies extrapolation factor F* (and hence the new estimate of potency in humans) would thereby be altered by a factor PK*/PK. The question is, what is the assumed value of PK?

7.4.1. Assuming that the Surface Area Correction Accounts for Pharmacokinetics

One approach is to take the case of the surface area correction factor of 12.7 between mice and humans (and hence the interspecies differences in a carcinogen's potency) as being entirely due to differences in pharmacokinetics (i.e., assume that PK = 12.7). A given tissue-concentration is taken to be equally toxic in all species (i.e., PD = 1). The rationale for this approach comes from the common pharmacokinetic observation that the volume of distribution of a compound tends to scale across species in proportion to body weight, while the rate at which the compound is cleared from that volume (by metabolism

and/or excretion) tends to scale as surface area. As a result, the concentration in the blood attenuates more slowly in larger animals, resulting in a larger area under the concentration-time curve, and presumably a larger biological effect (e.g., Dedrick et al., 1970). This approach is commonly used in scaling up doses of experimental drugs from mice to humans. Under this view, the reason that humans are assumed to be 12.7 times more sensitive than mice to a dose in mg/kg is that they experience an internal dose (area under the curve) that is 12.7 times higher.

If actual pharmacokinetic data for a certain compound showed that, contrary to this expectation, human internal doses were, say, one half as large as in mice following equal applied doses (i.e., PK* is observed to be 0.5), owing perhaps to some idiosyncracies of the compound's metabolism in humans, then this knowledge should prompt the lowering of the estimated human risk by a factor of $12.7/0.5 = 25.4$.

There are reasons to question whether the basis cited for this view of the surface area correction (the volume of distribution versus clearance argument) applies to carcinogens, however. Many carcinogens seem to require metabolic activation from a comparatively innocuous parent compound to a highly reactive metabolite. Thus, the concentration of parent compound and the rate of its clearance from the body may not be directly relevant to carcinogenic potency. For example, if the two means of clearance of a parent compound from the body, excretion and metabolism, both scale between species in proportion to surface

area, their ratio would be fairly similar in rodents and humans. Although human metabolism may be slower, the loss of parent compound by excretion is also slower to about the same degree. In other words, the same proportion of the dose may be metabolized for the compound in mice and humans, albeit over a longer time scale in humans. In this case, rather than 12.7, PK would be 1.0 between mice and humans, that is, the applied dose and internal dose are in the same proportion in different species. Of course, if metabolism and non-metabolic clearance do not remain in the same proportion, PK would assume some other value (for example, CEFIC studies are interpreted by the authors as showing that mice metabolize DCM by the GST pathway at least 60, rather than 12.7, times as fast as humans).

7.4.2. Assuming that the Surface Area Correction Accounts for Pharmacodynamics

If internal doses are assumed, as in the above example, to be generally proportional to applied doses, even across species, and if the overall extrapolation factor F is still assumed to be given by the surface area correction of 12.7, then the correction is being assumed to be due solely to species differences in the sensitivity of the tissues to carcinogenic transformation by a given internal dose, i.e., PD is assumed to be 12.7. A possible explanation for sensitivity scaling as surface area could be that some of the components of variation in tissue sensitivity, 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 different scales of "physiological time" among species, which tends to vary in proportion to body surface area. On the other hand, factors such as number of cells at risk would not scale in this fashion. A further complication is how to interpret the effect of a number of components each possibly scaling by surface area. Taken together, all of the components could lead to a factor of 12.7; alternatively, a multiplicative solution might be needed. For example, if each of the three components just listed caused a 12.7-fold effect, the net effect would be $12.7 \times 12.7 \times 12.7$, but if number of cells caused an opposite effect of 12.7×12.7 , the result would be a 12.7-fold effect. Obviously, the value of PD for any compound is subject to many considerations.

If, however, it is assumed that the surface area correction is applied in this way ($PD = 12.7$), it is a correction not on dose, but on risk from a given internal dose. The hypothetical compound mentioned above, for which pharmacokinetic data showed $PK^* = 0.5$, would, under this method, have only half of the pharmacokinetic difference expected by this view of the surface area correction to applied dose. Thus, the risk estimate would be lowered by a factor of $1/0.5 = 2$ (instead of by a factor of 24.5, as in the original example). When the surface area correction is viewed as a correction for tissue sensitivity differences across species, internal doses must be adjusted by the surface area correction just as applied doses are, because

the difference in sensitivity of the tissues between species is not addressed by pharmacokinetics.

7.4.3. Other Possible Assumptions for PD and PK

If there is an unusual pharmacokinetic pattern for a compound (i.e., PK* is shown to be different from its expected value, but PD retains the appropriate expected value), correction for species-to-species extrapolation should be made. A study of many compounds for which there are animal and human risk data, the purpose of which is to obtain estimates of PK*, may lead to clues regarding the values of PK and PD for this spectrum of compounds. If pharmacokinetics, for example, seems to always lower risks using 12.7 for PD as in the above example, the empirical success of applied dose scaling (which places some constraint on PK x PD) suggests that human sensitivity, PD, is perhaps being underestimated by the surface area correction.

One could start from a different set of assumptions about PK and PD. One cannot regard the use of pharmacokinetic information as replacing the need for assumptions about the "usual" scaling across species, since the scaling can always be determined empirically. This can only be done if a value of PD, the sensitivity differences between species, can be settled on. It is important to note that if one makes the decision to use pharmacokinetic data for species-to-species extrapolation, one cannot extrapolate across species using internal dose without making an assumption as to the value of PD. The approach described above is one attempt to show a derivation for PD.

Obviously, there is a large uncertainty in its value for a given chemical, and PD may vary considerably for other chemicals. When comparing risks extrapolated to humans on the basis of applied dose to risks extrapolated using internal or delivered dose, one must use the same value of PD (unless independent information on the biological basis of PD is also adduced to suggest that it too should be changed).

7.4.4. High- to Low-Dose Extrapolation

The preceding discussion focussed on the interspecies extrapolation, temporarily ignoring the fact that both PK and PD can change as a function of dose level. In the case of the pharmacodynamic factor, PD, the changes of response with internal dose are estimated by fitting a dose-response curve. The methods for doing this, and the assumptions that must be made, are essentially the same as when the dose measure is applied dose (the principal difference is that any influence of pharmacokinetic nonlinearities on the applied dose versus response curve is not falsely ascribed to pharmacodynamic differences with dose). These basic risk assessment methods are well discussed in federal regulatory agency risk assessment documents.

The relationship between the externally applied dose and the subsequent internal dose at the site of toxic action can vary with dose level because of saturation of metabolism, action of different biochemical pathways at different substrate concentrations, nonlinear patterns of binding of the compound,

and other factors. The assumption made by the applied dose procedure that similar biological processes underlie the action at low doses of a compound in animals and humans may be violated because the experimental animals are exposed to much higher dose levels. The equation describing the component assumptions of the applied dose extrapolation can be modified to

$$F = (PK \times HL) \times PD,$$

where the new factor, HL (for "high-to-low"), is the change in the ratio of human applied dose to internal dose between high doses (as high as rodents in the bioassay) and the low doses usually characteristic of the human exposures for which risk estimates are desired. In the applied dose procedure, HL is assumed to be 1; i.e., the possibility of such changes is ignored.

As a general principle, even very nonlinear pharmacokinetic systems usually approach linearity at low doses, below the level at which saturation effects are important. [The model used by Andersen et al. (1986, 1987) for instance, predicts GST metabolism that varies almost linearly with applied dose below about 10 ppm.] This is important because it means that the high-to low-dose extrapolation is the same for most exposure levels of concern (since they are all in the low-dose region), allowing a unit risk to be used in risk calculation. In other words, it is possible to calculate an observed value for the high- to low-

dose correction, HL*, which can replace the assumed value of HL = 1. Whether or not species-to-species extrapolation is employed, the HRAC agrees that nonlinearities in the dose-response curve due to pharmacokinetic differences must be accounted for in the risk assessment process, if the pertinent data are available.

HL is separable from PK and PD, and unlike the latter, has an assumed value for high- to low-dose extrapolation in the applied dose procedure--it is clearly assumed to be equal to one. Therefore, it is possible to incorporate pharmacokinetic information relating only to dose level into quantitative risk assessment. This method holds that the current lack of understanding of interspecies differences in tissue sensitivity (PD) precludes using internal doses to extrapolate across species, but assumes that the results of pharmacokinetic investigations can be used for high- to low-dose extrapolation; the pharmacodynamic factor is held constant within humans.

The extrapolation between species is done using the applied dose method. PK and PD need not be individually specified, only their product is fixed by whatever assumption the risk assessor has traditionally used (e.g., mg/kg/day or surface area correction). This method relies on the empirical success of applied dose extrapolation (Crump et al., 1985; Allen et al., 1986) discussed previously. These papers examine extrapolation only to high human doses, and in such cases the applied dose scaling factors work fairly well. The pharmacokinetic

information on humans is then used to define an observed non-proportionality (HL*) of applied and internal doses when high doses are compared to low doses. The extrapolation factor F is then adjusted to equal $PK \times PD \times HL^*$. This method accounts for nonlinearities in internal dose across exposure levels that arise from dose-dependent changes in absorption, disposition, excretion, and saturation or other changes in metabolism. However, it forgoes modifying the interspecies component of extrapolation on the basis of pharmacokinetics. New data changing the estimate of interspecies pharmacokinetic differences, PK*, will not result in different risks under this procedure.

The preceding pages have laid an extensive groundwork on the various factors that must be borne in mind when using pharmacokinetic information in the extrapolations inherent in quantitative risk assessment. The practical value of drawing the distinctions outlined above will be demonstrated in the next section, in which an analysis is presented of the interpretation by Andersen et al. (1986) of the results of their pharmacokinetic model for DCM vis-a-vis the EPA (1985b) and CPSC (1985) risk assessments.

7.5. THE PHARMACOKINETIC MODEL USED BY ANDERSEN ET AL. FOR DCM

Andersen et al. (1986) submitted to EPA and CPSC a report (which was subsequently published in 1987) on a physiologically based pharmacokinetic model along with interpretations of its results that, according to these authors, show the two agencies'

risk assessments to overestimate human risks from inhalation by 167-fold for liver tissue and 144-fold for lung tissue. The model used by Andersen et al. (1986, 1987) is a modification of an earlier model developed to describe the disposition of styrene (Ramsey and Andersen, 1984). The model, its merits, and its shortcomings have been discussed in Chapter 2. Here the intent is to analyze the contention of great overestimation of risks when extrapolation from mice to humans is done on the basis of applied dose instead of on internal doses as provided by the model. The model is used as presented by Andersen et al. (1986).

The HRAC finds that the differences between risk estimates derived from EPA/CPSC's applied dose method and from using internal doses from the model may be interpreted as being much smaller, only a few fold. The disagreement stems from two factors: (a) breathing rates, and (b) the appropriate application of the surface area correction to dose, as discussed previously.

7.5.1. Breathing Rates

In EPA's Addendum to the Health Assessment Document for DCM (1985b), and CPSC's risk assessment (1985), 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 Andersen et al.

(1986, 1987) incorporates the assumption that humans have a smaller input of DCM per kg than mice, but the model uses a set of breathing rates different from EPA's and CPSC's. The model's value for human breathing rate ($12.5 \text{ m}^3/\text{day}$) was measured for a man at rest, and is consequently much lower than EPA/CPSC's assumption ($20 \text{ m}^3/\text{day}$) 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.

Furthermore, the model's breathing rate value for mice ($0.084 \text{ m}^3/\text{day}$) is much higher than EPA/CPSC's assumption ($0.043 \text{ m}^3/\text{day}$). Andersen et al. (1986) 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 mice and for humans. One may debate about which set of rates is most appropriate, but the same set of values ought to be used when comparing the two methods. Since the breathing rate values appear both in the model and in the applied dose calculations, using the model's rates throughout implies a smaller human applied dose (and a bigger mouse applied dose) than the EPA/CPSC procedure does, but these applied doses are the ones that are truly associated with the model's estimates of internal dose. When the comparison is made in this way, using the Andersen et al. breathing rates in both procedures, the non-proportionality between applied dose and internal dose reported by Andersen et al. (1986) is reduced by a factor of 3.1, reflecting the different assumptions in the model and in the EPA/CPSC procedure

as to the ratio of mouse to human breathing rates. (The change in breathing rates in the EPA/CPSC procedure lowers the applied dose estimate, which is then less different from the model's results. If the EPA/CPSC breathing rates are used in both the model and the applied dose calculations, however, the model's results are not changed by 3.1-fold, but by a smaller amount, especially in mice, owing to nonlinear effects within the model. In a following section on developing a unit risk based on internal dose, the EPA/CPSC rates are in fact used.)

When the same breathing rates are used, the difference between the model and the applied dose procedure are not 167-fold and 144-fold for liver and lung, respectively, but rather 54-fold and 46-fold.

7.5.2. Using the Surface Area Correction

The second difference that the HRAC has with the analysis of Andersen et al. (1986) is in the use of the surface area correction on applied dose. Andersen et al. follow the first of the two interpretations of the surface area correction, as discussed in the earlier section. That is, they attribute to EPA/CPSC the assumption that, since the overall scaling factor for applied dose extrapolation across species is $F = 12.7$, the expected pharmacokinetic difference between mice and humans is $PK = 12.7$ (with $PD = 1$).

The model used by Andersen et al. (1986, 1987), using their "optimized" values for DCM kinetic constants (see previous sections for a discussion of the sensitivity of this procedure),

shows that the internal dose (metabolism by the GST pathway per liter of tissue) is not 12.7-fold greater in humans than in mice at the same applied dose, but is in fact somewhat lower in humans. In terms of the notation developed previously, the observed difference in the ratio of applied to internal dose when comparing mice at high doses to humans at low doses is $PK^* \times HL^* = 4.3$ for liver tissue, while in lung tissue the value is 3.6. [In liver, the interspecies extrapolation actually shows the applied dose method to underestimate the internal dose somewhat at 4000 ppm ($PK^* = 0.60$), but HL^* is 7.2, leading to a combined interspecies and high- to low-dose comparison of 4.3-fold; in lung, PK^* is about 1.6 and HL^* is 2.2.]

If the observed deficit of internal dose in humans vis-a-vis mice of 4.3-fold for liver is compared to the expectation of an excess of 12.7-fold, then the applied dose procedure overestimates internal dose (and therefore risk) by $12.7 \times 4.3 = 54$ -fold. In lung the calculation is $12.7 \times 3.6 = 46$ -fold. [If one adds back in the 3.1-fold inflation due to comparing different breathing rates, which is really extraneous to the comparison of applied to internal dose, then the factors are 167 and 144, as Andersen et al. (1986) originally reported them.]

If, on the other hand, one takes a second interpretation of the surface area correction--that the correction is to account for interspecies differences in sensitivity of the tissues to the internal dose as explained in the example above--then the assumed values of PK and PD are not 12.7 and 1, but rather 1 and 12.7,

respectively. The 4.3-fold deficit of internal dose in human liver vis-a-vis high-dose mice is thus compared to an expectation of 1. The change in the risk calculations is thus not 54-fold, but 4.3-fold, when internal doses are used in place of applied doses. The factor for lung is 3.6-fold, by the same argument. Since PD is assumed to be 12.7, the surface area correction is still applied to doses, even though they are internal doses, to correct for the anticipated greater sensitivity to those doses in humans compared to mice. If another value (other than 1.0 or 12.7) is assumed for PD, results would vary accordingly--one can see how important an assumption for PD is.

If one does not assume PD, but uses the high- to low-dose information only for incorporation of pharmacokinetic data into the risk assessment process, the difference between the Andersen et al. proposal and EPA/CPSC applied dose estimates is 7.2 or 2.2 for liver and lung, respectively (i.e., HL*).

The comparison by Andersen et al. assumes that the sole reason for applying the surface area correction factor of 12.7 to applied doses when extrapolating across species is to adjust for anticipated differences in pharmacokinetics between species. The HRAC feels that it is strongly arguable that the surface area correction is not a correction on dose to allow for pharmacokinetics, but rather a correction on risk to allow for many factors, including pharmacodynamics. The HRAC's interpretation of the results of the model used by Andersen et al. (1986, 1987) is that, although it indicates some

overestimation of internal dose of GST metabolism in humans compared to the level in mice in the NTP bioassay, this effect can easily be only a few fold.

Nonetheless, it is important to judge the impact of such results on the DCM risk assessment process. The following section develops a unit risk for DCM based on extrapolating risks from mice to humans on the basis of GST metabolism in liver and lung tissue.

**7.5.3. Developing a Unit Risk Based on Internal Dose:
Incorporation of High- to Low-Dose Differences and
Species-to-Species Differences**

This section outlines the development of a unit risk based on the amount of metabolism by the GST pathway, as estimated by the model used by Andersen et al. (1986, 1987). It was assumed that $PD = 12.7$, as outlined in the above example, i.e., that the carcinogenic potency of DCM in corresponding organs of mice and humans is assumed to be equal when the daily amount of metabolism by the GST pathway per liter of target organ tissue (the "internal dose") is scaled by body weight to the $2/3$ power, which is proportional to the relative surface area to volume ratio of mice and humans. This is accomplished by dividing the output of the model for humans by a factor of 12.7 before risks are calculated to account for a presumed difference between mice and humans in tissue sensitivity to a given tissue-level dose. (Surface area scaling could be based on organ weight rather than on body weight, but as organ weights are nearly a constant

proportion of body weight, this would make little difference in the eventual unit risk, a difference of only 3% greater for lung and 8% greater for liver.)

Lung and liver do not generate equal amounts of metabolites during exposure, so it is necessary to extrapolate risks for each organ separately. (This exercise does not imply that the HRAC's position is that humans and animals must respond at similar sites. The HRAC provides this example for methodological illustration; this problem is discussed further below.) Separate incidence rates of lung and liver tumors in female mice (the more sensitive sex) were drawn from the NTP (1985) bioassay. For hepatocellular adenomas and/or carcinomas the incidences of tumor-bearing animals were: 3/45 in the control group, 16/46 in the 2000 ppm group, and 40/46 in the 4000 ppm group; for alveolar-bronchiolar adenomas and/or carcinomas the incidences were 3/45 among controls, 30/46 at 2000 ppm, and 41/46 at 4000 ppm. Animals dying before the appearance of the first tumor (which occurred in week 68 for both tumor types) have been eliminated from the denominators, since the capacity of these individuals to develop tumors is not fully tested.

Internal doses were estimated using the model used by Andersen et al. as presented in Andersen et al. (1986), with the following exception: the HRAC feels that the federal regulatory agencies' 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 used in the model were adjusted accordingly. Cardiac output was adjusted by the same proportion as the breathing rates. These changes represent less a modification of the model than a change in the assumption about the activity level at which the model is to estimate internal doses.

The modified mouse pharmacokinetic model was used to estimate internal doses under the exposure regime of the NTP bioassay, that is, 6 hours a day at 2000 or 4000 ppm. The results showed that virtually no DCM remained in the body after the 18 hours following one exposure and preceding the onset of the next day's exposure, so accumulation of compound in the body over time is not an issue in this case. The internal doses resulting from a day's exposure were multiplied by 5/7 to provide an average daily internal dose (since dosing was for 5 days per week). The resulting values are 727.8 and 1670 mg/L for liver and 111.4 and 243.7 mg/L for lung in the low- and high-exposure groups, respectively.

These internal doses, along with the corresponding tumor incidences, were then used to construct dose-response curves based on the multistage model procedure, using the computer program GLOBAL86 (Howe et al., 1986). The liver data were fitted by a two-stage model, while a one-stage model was fitted to the lung tumor data. (The number of stages was chosen as that number from 1 to 6 leading to a model with the minimum q_1^* , the 95% upper bound on the estimated linear term. Since q_1^* is used as the basis for low-dose extrapolation, all alternative possibilities

for the number of stages in the model would result in higher risk estimates.)

The extrapolation of the observed risks at high doses to those estimated at low doses was accomplished using the 95% upper bound on the fitted curve. This corresponds to choosing the curve with the largest linear component (which will dominate the low-dose shape of the curve) subject to the constraint that the curve fits the data reasonably well in the observed range. This process reflects the difficulty in determining the shape of the dose-response curve at low doses. Most conceptions of the carcinogenic process lead to the expectation that, at worst, risk should decrease in direct proportion to decreasing dose at sufficiently low doses. If the true dose-response curve is convex at low doses, actual low dose risks will be less, perhaps much less, than those estimated by the upper-bound curve.

Next, the human pharmacokinetic model was used to estimate the internal doses from a continuous exposure to a low dose [1 ppm, as used by Andersen et al. (1986)]. The rate of metabolism by the GST pathway under such an exposure is 0.07011 mg/L/day in liver and 0.008386 mg/L/day in lung. These internal doses were modified by the surface area scaling described previously, and the risks that result from such tissue-level exposures were estimated from the curve of internal dose versus response developed from the female mouse tumor data. These risks give the lifetime probability of developing cancer from continuous exposure to 1 ppm, under the stated set of

assumptions. Because internal dose varies directly with vapor concentration at such low exposure levels, and because (by assumption) risk is taken to vary directly with internal dose, the estimated risks at 1 ppm can be used as unit (or incremental) risks expressed in units of vapor concentration. When these unit risks are converted to units of $(\mu\text{g}/\text{m}^3)^{-1}$, they are 1.34×10^{-7} for liver and 3.33×10^{-7} for lung.

The risk based on mouse lung tumors is a bit over twice that based on mouse liver tumors. In fact, however, the risk in lung tissue per unit of internal dose is about 20-fold higher than for liver tissue, but for any given exposure to DCM vapor, the liver has a much higher internal dose, owing to its greater metabolic activity, so that overall risks in the two tissues are nearly comparable. Small errors in the model's allocation of GST metabolism between liver and lung could have large consequences on risk estimates, since metabolism in the lung evidently engenders much more risk.

The above phenomenon illustrates a difficulty that arises in interspecies extrapolation when tissue-specific doses and risks are calculated. If one expects strict site concordance across species, then the liver and lung unit risks estimate the organ-specific risks in humans. But the tissue-specific extrapolations run into difficulty when they are used to predict an overall level of human cancer risk, which may be manifested in other organs beside liver or lung. For example, it is problematic to arrive at a prediction of possible risk to human pancreas, given

that there is no internal dose estimate for this organ, nor any information on tissue-specific risk from a given internal dose (which can vary considerably, as shown by the mice).

In mice, the occurrence of liver and lung tumors is independent; developing a tumor at one site does not affect the probability of developing a tumor at the other site. If this is also true in humans, then overall estimates of total cancer risk from DCM exposure are given by the sum of the liver- and lung-based risk extrapolations. Thus, one can arrive at an overall unit risk by simply adding the tissue-specific unit risks. Adding the individual unit risks yields a human unit risk for continuous inhalation exposure to 1 mg/m^3 of 4.7×10^{-7} . This unit risk is 8.8-fold lower than the EPA's (1985b) published unit risk based on applied dose (4.1×10^{-6} per ug/m^3), which is based on the same bioassay data.

If it were decided that the surface area correction ought not be applied to internal doses before risk calculation, the procedure would be the same as outlined above, with the sole exception that the division of human internal dose by 12.66 before risk calculation would not be done. Omitting this step corresponds to the assumption that corresponding human and mouse tissues are equally sensitive to carcinogenic transformation by a given internal dose. The unit risk calculated in this way would be lowered by 12.66-fold from that calculated above. It would thus be 111-fold less than the unit risk in EPA's applied dose analysis (U.S. EPA, 1985b). On the other hand, if PD turns out

to be 100, there would be no difference in the applied versus internal dose-based estimated risks.

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. The HRAC's analyses of the model used by Andersen et al. (1986, 1987) indicate that, so long as vapor concentrations remain low (below 100 ppm), single exposures, intermittent exposures, and exposures to varying vapor concentrations, are all nearly equivalent in the internal doses they produce to a continuous exposure to DCM at the time-weighted average level. In other words, noncontinuous and other-than-lifetime exposures can be converted to lifetime average daily equivalent exposures before risks are calculated.

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 disproportionately 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 m³/day. Occupational exposures, or other exposures occurring during more-strenuous-than-average activity, will

consequently have risks somewhat underestimated.

7.5.4. Developing a Unit Risk Based on Internal Dose:

Incorporation of Only High- to Low-Dose Differences

Because of the current lack of data bearing on the question of interspecies differences in tissue sensitivity to carcinogens, it is exceedingly difficult to construct a sound argument as to why a particular value of PD should be settled upon. Not only is it difficult to argue for a PD value of 12.7 versus 1, as outlined above, but other more widely ranging values are possible as well, constrained only by the limited data showing that the product $F = PK \times PD$ is in the neighborhood of between surface area scaling of dose and body weight scaling of dose (Allen et al., 1986).

Furthermore, there is a great deal of uncertainty in the estimates of internal dose generated by the model used by Andersen et al. (1986, 1987), as discussed earlier in this document. In view of these uncertainties, it is wise to take careful account of the metabolism data from all sources, and to define a set of conclusions that seem the most robust despite the unavoidable uncertainties. Then, the extrapolation of risk from high-dosed mice to humans can be examined in the light of what can be concluded from these findings, from the viewpoint of using those pharmacokinetic data which, due to a strong weight-of-evidence indication, the HRAC feels should at a minimum be incorporated into assessments of risk for DCM. The following section reviews the data and weight of evidence, and then

develops an extrapolation of risk to humans that uses the most robust conclusions about pharmacokinetics to extrapolate from high human doses to low doses, accounting for the effect of saturation of the MFO pathway at high exposure levels.

7.5.4.1. Review of Metabolism Data--As discussed previously, DCM is metabolized by two known pathways: the MFO pathway leading to carbon monoxide as an end product, and hypothesized by some to lead to carbon dioxide as well, and the GST pathway, leading to carbon dioxide as the end product. The available data, as thoroughly discussed in previous chapters, indicate that the MFO pathway is saturable in animals at levels below the tested inhalation levels in the NTP bioassay. Based on the exhaustive data base in animals and what little human data are now available, it is likely that this pathway saturates in humans as well. The available data, however, indicate that the GST pathway is not saturated, even at the highest levels tested in the NTP bioassay (the term "non-saturating" applies up to this exposure level; obviously, the pathway will saturate at much higher levels). Based on new in vitro data just submitted to HRAC, this would seem to be the case in humans as well. At higher levels, once the MFO pathway saturates, whatever output occurs from the GST pathway for any dose increment is likely to be linearly dose dependent, due to the projected first order kinetics of the GST pathway even at high dose levels; this approach would not be inconsistent with the applied dose method at high doses (both are linear at high doses in the case of DCM). However, at lower

doses, where the MFO pathway is not saturated, the amount of parent compound available to the GST pathway, relative to applied dose, would be less than that at MFO--saturating levels--for the obvious reason that for any dose increment, the MFO pathway removes some of the parent compound that would otherwise be available to the GST pathway. Thus, given that the MFO pathway saturates, a nonlinear output from the GST pathway is expected at low (non-saturating) doses relative to higher (saturating) doses.

It is thus important to elucidate the role of these two pathways with regard to the carcinogenic potential of DCM. In doing so several possibilities must be considered:

- (1) The GST pathway is responsible for the carcinogenic response
- (2) The MFO pathway results in the production of carbon monoxide and possibly (hypothesized) in the production of carbon dioxide. One or the other of these routes (or both) is responsible for the carcinogenic response.
- (3) The parent compound is responsible for the carcinogenic response.
- (4) Some combination of the above is responsible for the response.

The saturation of the MFO pathway is based on results looking for carbon monoxide generation. According to the recently available DCM data for model input, and PBPK models developed for DCM, this portion of the MFO pathway is expected to be saturated not only in the NTP inhalation bioassay at all dose levels in mice, but also in the National Coffee Association (NCA)

drinking water study at the highest dose level in mice.

[According to the model used by Andersen et al. (1986, 1987), MFO pathway output is an average of 2550 and 3200 mg DCM metabolized/liter of tissue/day for the NTP low dose and NCA high dose, respectively.] If the carbon monoxide portion of the MFO pathway were of primary importance, a response similar in magnitude to that observed in the NTP bioassay would have been expected at the highest dose of the NCA study, since the output of this saturable pathway is estimated to be approximately the same in these two cases. However, this was not observed; the response in the NCA study was nonsignificantly elevated, much smaller than the NTP response, but of a magnitude consistent with a linear extrapolation from the higher NTP doses. Also, a dose-response relationship for lung and liver would not be expected to be as readily apparent as seen in the NTP bioassay, if the MFO pathway was of primary importance, since again this pathway is expected to be saturated, with a similar output, at both doses. [The model used by Andersen et al. (1986, 1987) predicts MFO output as an average of 2550 and 2650 mg DCM metabolized/liter of tissue/day for the NTP low and high doses, respectively.] This argument does not eliminate the MFO pathway from consideration with regard to a role in the carcinogenic response of DCM, but it does indicate that some other pathway or chemical species is likely to be of greater importance.

This leads to the likelihood that the GST pathway is of primary importance for the carcinogenic response. The GST

pathway intermediates are easily envisioned as being able to interact with the genetic material, supporting an important role for this pathway. Short-term tests indicate that DCM itself must be metabolized before genotoxic effects are observed; although this does not eliminate the parent compound from having a role, it emphasizes a potential role for GST metabolites. In this case, estimated risks at lower doses, especially those below the point at which the MFO pathway saturates, would reflect the nonlinear output of the GST pathway as described in the beginning of this section, since they are based on observed risks at higher doses.

However, even if there is a nonsaturable portion to the MFO pathway (e.g., leading to carbon dioxide) which has a role in carcinogenesis, 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.

Thus, the HRAC believes that, based on the weight of evidence, some adjustment using pharmacokinetic data should be made when extrapolating from high to low dose. As explained previously in this section, however, the HRAC at this time is unsure about how to use pharmacokinetic data that indicate metabolic differences between species. This is because

extrapolation between species involves many factors, one of which is metabolism/pharmacokinetics. The ability to elucidate one component of a species difference does not necessarily indicate what, if any, adjustments should be made; it does not provide more certainty than the empirical process currently used. Thus, in the following section the HRAC performs an analysis of how high- to low-dose effects on metabolism may affect ultimate risk.

7.5.4.2. Robustness of Model Output--In the case of DCM, the HRAC has examined the effects on GST output in the lung and liver, using the model used by Andersen et al. (1986, 1987), relative to dose. As explained elsewhere in this document, the model is generally able to estimate levels of DCM in various situations, such as chamber disappearance, blood levels, etc. Thus, the model is viewed to be reasonably able to predict levels of DCM in tissues such as blood, lung, and liver. As explained elsewhere as well, the model seems to be sensitive to changes in the metabolic constants k_F , K_M , and V_{max} .

At the values that Andersen et al. (1986, 1987) estimated for these three constants in humans (k_F , 0.53; K_M , 0.58; V_{max} , 118.9), the nonlinear contribution to the projected human dose-response curve due to consideration of DCM pharmacokinetic data is 2.2 for the lung and 7.2 for the liver. These numbers are derived by dividing the outputs of the model used by Andersen et al. [RISK2L or RISK2P: measures of the output of the GST pathway; the 2 stands for the GST pathway, and the L or P stands for liver or lung (pulmonary), respectively] at 4000 ppm by

RISK2L or RISK2P at 1 ppm, and then dividing that by 4000 to get the difference relative to dose.

Having established that some correction, i.e., HL*, to "target" dose levels due to high- to low-dose extrapolation (the factors 2.2 and 7.2 are examples of such a correction) is necessary, it is important to determine the sensitivity of these ratios as the underlying metabolic constants input into the model are varied. In other words, even though the absolute output of the model is sensitive to metabolic constant variation, the relative output (e.g., factors such as 2.2 and 7.2) may not be. This is important in the determination of the magnitude of the correction the HRAC recommends to be applied on the basis of pharmacokinetic data.

Table 12 displays the results of a sensitivity analysis for k_F based on varying the metabolic constants input into the model used by Andersen et al. (1986, 1987) for DCM. The ratio is, again, RISK2L or RISK2P at 4000 ppm divided by RISK2L or RISK2P at 1 ppm, and then divided by 4000 to get a ratio relative to dose. The ratio is relatively insensitive to the value of k_F , the GST pathway metabolic constant. Thus, even if k_F is off by more than an order of magnitude, no real change in the ratio is expected. (The value of 4-fold was chosen based on the sensitivity analysis performed on k_F elsewhere in this document; the value of 10-fold to look at order of magnitude differences, and the value of 53-fold lower to approximate the possible reduction in k_F based on the CEFIC data; see previous sections

for a review of these data.)

TABLE 12. SENSITIVITY ANALYSIS FOR k_F

| k_F | K_M | V_{max} | Comments | Ratio | |
|-------|-------|-----------|-------------------------|-------|-------|
| | | | | Lung | Liver |
| 0.01 | 0.58 | 118.9 | k_F decreased 53-fold | 2.3 | 7.6 |
| 0.053 | 0.58 | 118.9 | k_F decreased 10-fold | 2.3 | 7.4 |
| 0.13 | 0.58 | 118.9 | k_F decreased 4-fold | 2.3 | 7.4 |
| 0.53 | 0.58 | 118.9 | Model values | 2.2 | 7.2 |
| 2.12 | 0.58 | 118.9 | k_F increased 4-fold | 2.1 | 6.5 |
| 5.3 | 0.58 | 118.9 | k_F increased 10-fold | 1.9 | 5.5 |

Tables 13 and 14 look at similar analyses for V_{max} and K_M , the MFO pathway metabolic constants. Again, the lung ratios are not too dissimilar from the ratio using the Andersen et al. values when varied by an order of magnitude (only at a 10-fold decrease does the ratio start to approach no difference as opposed to an approximate 2-fold difference); the liver values

TABLE 13. SENSITIVITY ANALYSIS FOR V_{max}

| k_F | K_M | V_{max} | Comments | Ratio | |
|-------|-------|-----------|-----------------------------|-------|-------|
| | | | | Lung | Liver |
| 0.53 | 0.58 | 11.89 | V_{max} decreased 10-fold | 1.4 | 1.7 |
| 0.53 | 0.58 | 29.70 | V_{max} decreased 4-fold | 1.7 | 2.7 |
| 0.53 | 0.58 | 118.9 | Model values | 2.2 | 7.2 |
| 0.53 | 0.58 | 475.6 | V_{max} increased 4-fold | 2.2 | 20 |
| 0.53 | 0.58 | 1189 | V_{max} increased 10-fold | 1.8 | 32 |

TABLE 14. SENSITIVITY ANALYSIS FOR K_M

| k_F | K_M | V_{max} | Comments | Ratio | |
|-------|-------|-----------|-------------------------|-------|-------|
| | | | | Lung | Liver |
| 5.3 | 0.058 | 118.9 | K_M decreased 10-fold | 2.7 | 63 |
| 5.3 | 0.145 | 118.9 | K_M decreased 4-fold | 2.6 | 26 |
| 5.3 | 0.58 | 118.9 | Model values | 2.2 | 7.2 |
| 5.3 | 2.32 | 118.9 | K_M increased 4-fold | 1.6 | 2.5 |
| 5.3 | 5.8 | 118.9 | K_M increased 10-fold | 1.3 | 1.6 |

vary more considerably, but interestingly, the ratio does not fall much below two over this 100-fold range.

A final analysis (Table 15) looks at changes in both K_M and V_{max} , but altering them simultaneously by the same magnitude (looking at the sensitivity of the ratio of V_{max}/K_M). The ratio is much less sensitive to simultaneous changes in V_{max}/K_M than to either variable alone. Consideration of this type of analysis is important in cases where K_M is large enough that the rate of response for the MFO pathway is essentially proportional to V_{max}/K_M , in effect, a first-order rate constant somewhat like k_F [since for a saturating system, rate is related to $V_{max} \cdot C / (K_M + C)$, where C is concentration; at high K_M relative to C , the equation can be approximated as $V_{max} \cdot C / K_M$, and thus the rate at various concentrations is essentially related to V_{max}/K_M].

The HRAC interprets the above analysis to indicate that even if the Andersen et al. constants are in error by an order of magnitude in either direction, the ratio of metabolism by the GST pathway at low versus high dose, relative to dose, will always be

TABLE 15. SENSITIVITY ANALYSIS FOR V_{max}/K_M

| k_F | K_M | V_{max} | Comments | Ratio | |
|-------|-------|-----------|---------------------------------|-------|-------|
| | | | | Lung | Liver |
| 5.3 | 0.058 | 11.89 | V_{max}/K_M decreased 10-fold | 2.4 | 7.8 |
| 5.3 | 0.145 | 29.70 | V_{max}/K_M decreased 4-fold | 2.3 | 7.8 |
| 5.3 | 0.58 | 118.9 | Model values | 2.2 | 7.2 |
| 5.3 | 2.32 | 475.6 | V_{max}/K_M increased 4-fold | 1.9 | 5.7 |
| 5.3 | 5.8 | 1189 | V_{max}/K_M increased 10-fold | 1.6 | 4.1 |

decreased by a factor of, at least, approximately two for either lung or liver. Furthermore, the analysis may be able to tolerate even more error. The ratio is least sensitive to k_F ; variation of this constant by nearly two orders of magnitude results in little variation of the ratio. The value, k_F , according to Andersen et al. (1986), is the only constant that was truly optimized and scaled for humans; V_{max} and K_M were based on human data (which are currently being analyzed by the HRAC), which ostensibly are subject to less error.

7.5.4.3. Using Pharmacokinetics for High- to Low-Dose

Extrapolation--Given the above sensitivity analysis, the HRAC has developed the following procedure for the calculation of human risk due to exposure to DCM.

- (1) Calculate human doses to be input into a risk assessment model from animal dose data by whatever species-to-species conversion factor has been conventionally used (e.g., mg/kg/day, surface area correction).
- (2) Modify these human doses by dividing the applied dose by the appropriate factor derived from human lung GST metabolism.

model from animal dose data by whatever species-to-species conversion factor has been conventionally used (e.g., mg/kg/day, surface area correction).

- (2) Modify these human doses by dividing the applied dose by the appropriate factor derived from human lung GST metabolism according to the model used by Andersen et al. (1986, 1987). The equation to derive the factor for a given dose X is:

$$\text{Factor} = \text{RISK2P4000} * X / (\text{RISK2PX} * 4000)$$

where RISK2P4000 is RISK2P at 4000 ppm and RISK2PX is RISK2P at X ppm.

- (3) Use these doses, and the animal responses, for input into the mathematical extrapolation model. When using the output of the mathematical extrapolation model to calculate risk for a specific applied environmental dose, apply the above factor to the environmental dose before using the model to predict a risk for that dose. Realize that the pharmacokinetic model should be run to account for duration of exposure.
- (4) All other calculations, such as proportion of lifetime exposed, remain unchanged.

This procedure allows the incorporation of a factor, due to consideration of high- to low-dose pharmacokinetic differences in the case of DCM, of between 1.0-fold (at high doses) and about 2.2-fold (at low doses). The factor may indeed be greater, but

the HRAC does not recommend that this procedure, for a minimum pharmacokinetic-based adjustment, go beyond the limits of the data as currently available. However, the weight of evidence does justify, in the opinion of the HRAC, reducing the upper bound or any other DCM risk estimate based on applied dose by the aforementioned factor, at minimum. Furthermore, the HRAC realizes that if some other tissue, such as the pancreas, is a target, the above rationale would still hold since saturation of the MFO pathway is still a very likely hypothesis for any tissue, and the GST pathway is found in many tissues, including the pancreas (Mukhtar et al., 1981). Finally, as in the approach assuming $PD = 12.7$, HL^* 's can be calculated if no exposure is above 100 ppm, and thus lifetime average daily equivalent exposures can, in such situations, be calculated before mathematical risk assessment models are employed.

7.6. CONCLUSIONS

It is clear that, once estimates or measurements of internal dose at the sites of toxic action are obtained, there are still many difficult issues to be faced in deciding 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 faults in the information on pharmacokinetics for this compound. It is a general problem, reflecting the lack of understanding of the pharmacodynamics of carcinogenesis.

The use of pharmacokinetics only for high- to low-dose extrapolation, outlined above, has the advantage that it is quite

insensitive to the major uncertainties in the pharmacokinetic data. This method accounts for the nonlinearities in internal dose across exposure levels that arise from dose-dependent changes in absorption, distribution, excretion, and saturation of metabolism. The principal effect is the nonlinearity of GST metabolism between high and low doses as a result of saturation of the competing MFO pathway, which is relatively well characterized. The method forgoes using pharmacokinetics for interspecies extrapolation, due to uncertainty in assessing the impact of a given internal dose difference in view of the lack of knowledge of sensitivity differences. It thereby avoids the question of the relative contribution of pharmacokinetics and pharmacodynamics to interspecies scaling of carcinogenic potency, but it assumes that the combined effect of these (the product PK x PD) is more or less reliably given by cross species extrapolation based on applied dose. This method results in a minimum lowering of the implied risk to humans that seems necessary in view of the data. If data became available indicating that human GST metabolism is much lower than previously estimated, the risk calculation by this method would not change, since the interspecies difference in potency is not informed by metabolic differences between species (although such differences clearly have an effect on potency, the method assumes that the effect cannot be estimated).

The use of pharmacokinetics for interspecies extrapolation as well as high- to low-dose extrapolation, discussed earlier in

this chapter, does incorporate interspecies differences in metabolism, and changes in this comparison will change the estimates of human risk accordingly. 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, at least provisionally, in the estimation of human risk. To make the interspecies extrapolation, however, this method must make a further assumption about the relative differences in tissue sensitivity to internal doses between experimental animals and humans. That is, one must make an assumption about the value of PD, and not just about the product $PK \times PD$. Uncertainty in the proper value for species differences in pharmacodynamics leads to widely divergent risk estimates. This method is also more sensitive to errors in the pharmacokinetic model revolving around the determination of k_F (see previous sections).

Both methods must assume that the relationship of internal dose to risk within species is adequately characterized by the process of fitting dose-response curves and extrapolating them to low doses. The shape of the dose-response curve at low doses depends heavily on the mechanism of action involved in DCM's carcinogenicity. This mechanism is very poorly understood, and the uncertainties about low-dose extrapolation of pharmacodynamics probably greatly exceed those about interspecies extrapolation of potency.

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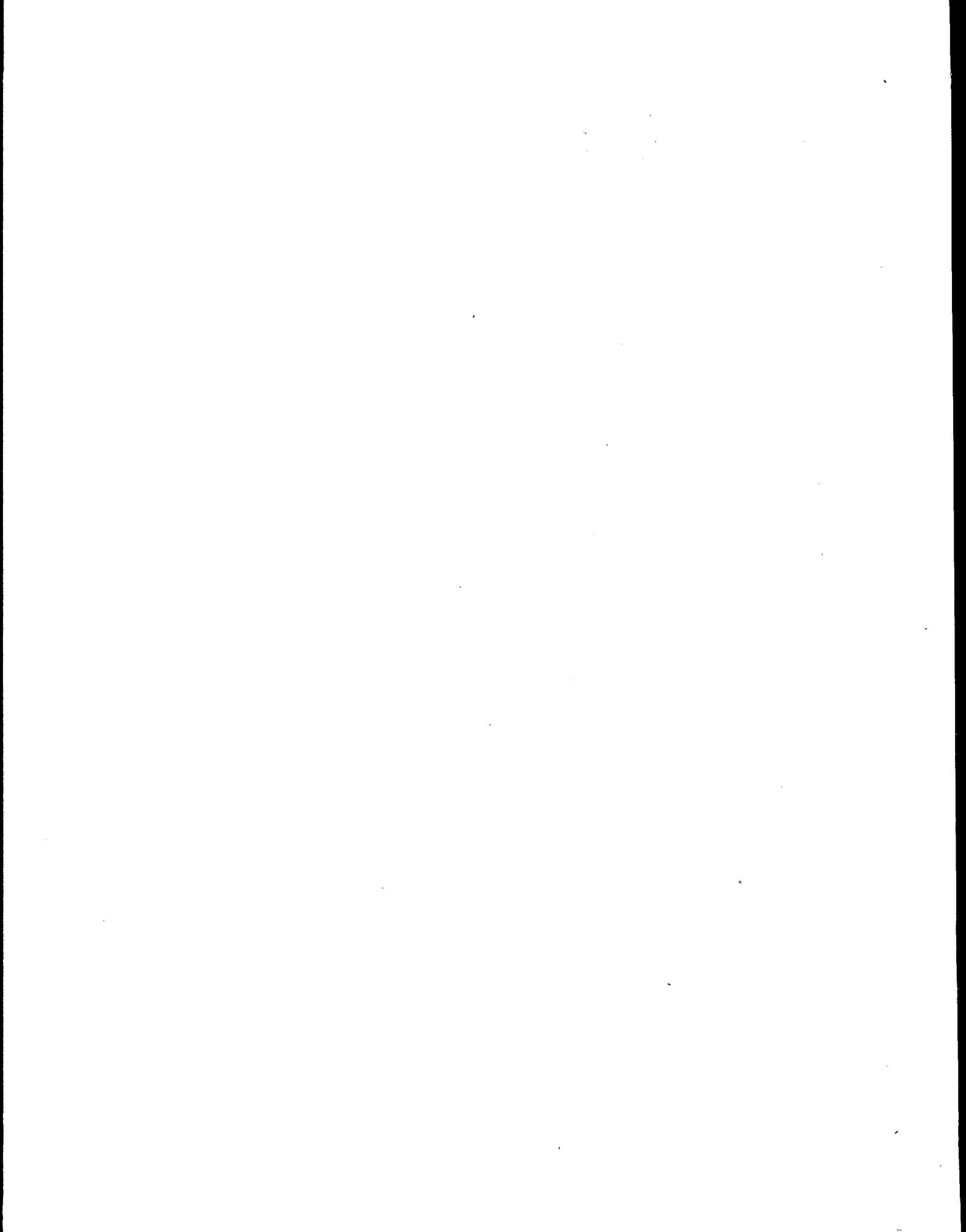
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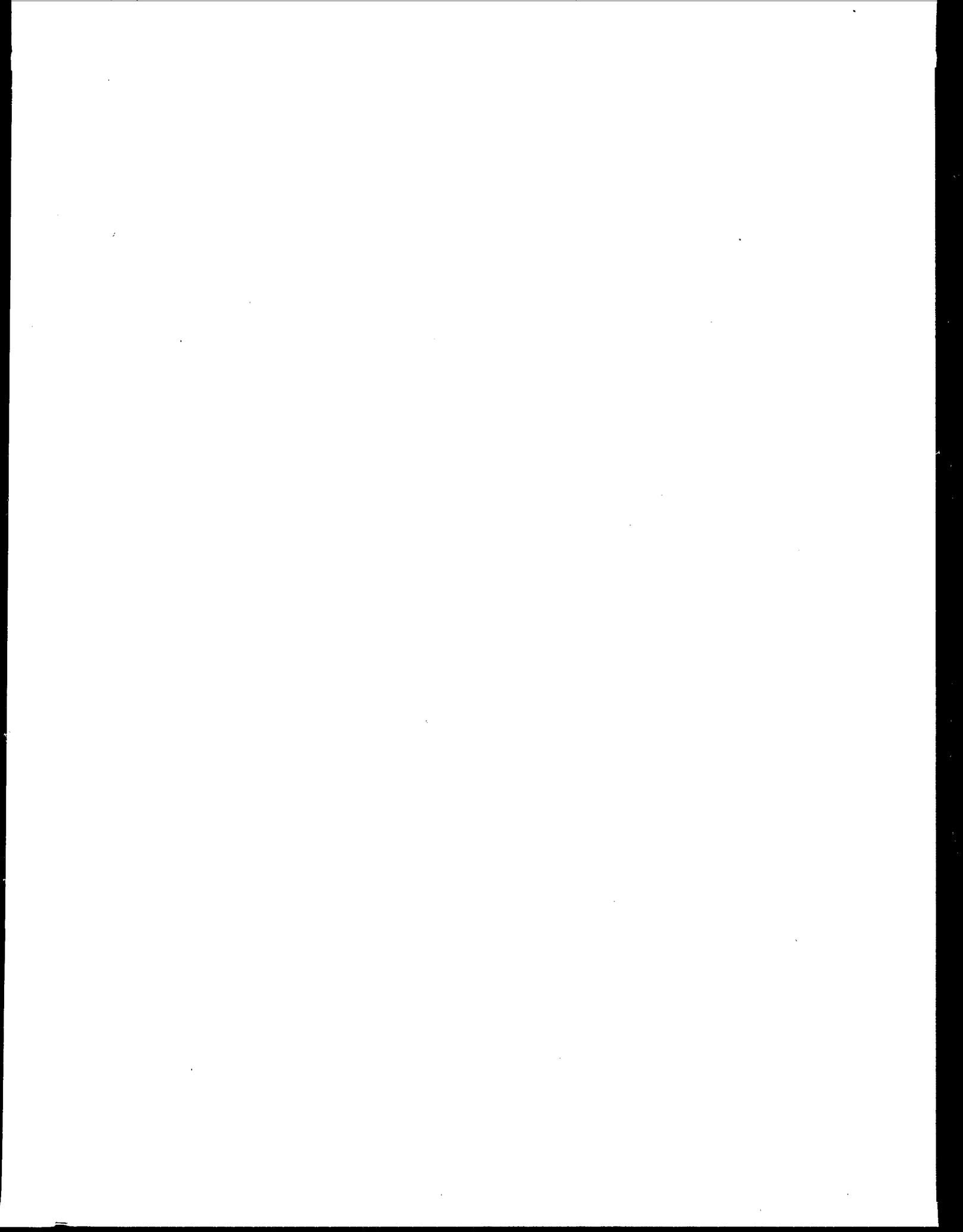
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