An Approach to Mechanism-based Cancer Risk Assessment for Formaldehyde

by Rory B. Conolly and Melvin E. Andersen

The established carcinogenicity of formaldehyde in the rat and suggestive epidemiological evidence that formaldehyde may be a human carcinogen have led to its regulation by U.S. Federal agencies as a probable human carcinogen. These risk assessments have typically been based on tumor data in F344 rats exposed chronically to formaldehyde by inhalation and used the inhaled concentration as a measure of dose and the linearized multistage model (LMS) for dose–response characterization. Low-dose risks estimated with the LMS are thought to be conservative but are also generally acknowledged to be highly uncertain. In this manuscript, we first consider in generic terms how use of chemical-specific data on mechanisms of target tissue dosimetry and the series of tissue responses to the chemical that culminate in tumor formation can lead to more accurate dose–response characterization. A planned mechanism-based risk assessment for formaldehyde is then described. This risk assessment uses data on target tissue dosimetry, size of the target cell population in the rat nasal epithelium, number and size of putative preneoplastic lesions, and tumor incidence. These data establish parameter values for a biologically based, multistage cancer model that is then used to predict cancer risk at low exposure levels. Such work provides insights into the relative roles of formaldehyde-stimulated cell replication and procarcinogenic mutation in tumor formation. Finally, future directions are outlined for research on tissue dosimetry and scaling of the mechanism-based formaldehyde risk model from rats to people.

Introduction

Formaldehyde is a commercially important chemical and a common air contaminant in the workplace and the home (1–4). Although formaldehyde is a primary irritant (5,6) and may be a sensitizer in a subset of the population (7), its potential carcinogenicity is the health effect of greatest concern. Formaldehyde causes squamous cell carcinoma in the nasal cavity of rats and mice exposed chronically to relatively high concentrations (6) (Table 1). Human epidemiological studies are equivocal as to whether long-term formaldehyde exposure is associated with respiratory tract cancer (8,9). When epidemiological evidence is unequivocal and includes sufficient information on the exposure–response relationship, human cancer risk assessment may be based on the epidemiological data rather than on animal studies. Benzene has been regulated in this manner (10). The epidemiological data for formaldehyde, however, are not by themselves sufficient to support a quantitative cancer risk assessment (8,9). Formaldehyde risk assessment has thus been based on data obtained from animal experiments. The U.S. EPA published a can-

cer risk assessment for formaldehyde in 1987 (11) and has recently considered a revised risk assessment incorporating data on tissue dosimetry (9).

The purpose of this manuscript is to provide a critical review of the use of the linearized multistage (LMS) model in the current approach to cancer risk assessment to examine how this approach can be improved by including data on the mechanisms linking exposure with carcinogenic response and to outline how this mechanistic approach will be used with formaldehyde. For our purposes, "mechanism" refers to how an event takes place. The events of interest are a) the disposition of toxic chemicals throughout the body and especially to the target tissue(s), b) the initial biochemical interaction between the chemical and target tissue, and c) the tissue response characterized by progressive cellular alterations leading to frank tissue toxicity, carcinogenicity, or any of a variety of end points. The interactions among physiochemical,

Table 1. Nasal cavity squamous cell carcinoma incidence in formaldehyde-exposed rats.[a]

Formaldehyde concentration, ppm[b]	Tumor prevalence
0	0/160
2.0 ± 0.01	0/160
5.6 ± 0.02	2/160
14.3 ± 0.04	87/160

[a]From Starr and Buck (29). Number of tumor-bearing animals/number of animals considered to be at risk.
[b]Mean ± SE over 24 months.

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physiological, biochemical, and molecular factors provide the mechanistic basis of disposition, toxicant-target tissue interaction, and toxic effects in an animal species at various dose levels of a chemical. The interplay of all these factors determines how a particular process takes place. The actual mechanism-based formaldehyde risk assessment is not presented.

Default Approach to Cancer Risk Assessment

Risk assessment is a multistep process involving hazard identification, dose–response assessment (including animal to human extrapolation), exposure assessment, and risk characterization (12). (The dose–response assessment is more appropriately defined as the exposure–response or exposure–dose–response assessment.) Our efforts focus on the exposure–response characterization, as this component of the overall risk-assessment process is ripe for significant modification by inclusion of mechanistic data.

In the current standard approach to cancer risk assessment, correlational data on the relationship of exposure to tumor response are obtained in a rodent bioassay and are used, along with a series of default assumptions, to predict human health risk. For example, the formaldehyde risk assessment conducted by the EPA (11) used data on the tumor response in F344 rats exposed chronically to 2.0, 5.6, or 14.4 ppm formaldehyde (Table 1). People are usually exposed to much lower levels of formaldehyde. Prediction of the shape of the tumor response curve expected in people for the lower, more relevant exposure levels is thus necessary. The EPA (11) prediction was obtained with the LMS model, a polynomial function whose parameters are adjusted to obtain an exposure–response curve that fits the tumor data and predicts a nonzero response for all nonzero exposure levels (13,14). The results are typically presented as the upper 95% confidence bound on the risk estimate rather than as a mean value for a given exposure level.

Low-dose risk predictions using the LMS model are generally thought to be conservative and protective of the public health. However, the LMS does not use mechanistic data specific to the chemical being regulated, and one cannot state with any certainty the degree to which the shape of the curve predicted by the LMS for the low-dose region is a true representation of the real-world curve. A LMS-based risk assessment may be overly conservative, engendering unnecessarily stringent regulation and correspondingly high costs for exposure control. On the other hand, there is also some presumably small possibility that true risk is underestimated by ignoring mechanistic data. In the following section we consider how the uncertainties in low-dose extrapolation encountered with the LMS model can be reduced by explicit consideration of the biological mechanisms that link exposure with carcinogenic response.

Mechanism-based (Biologically Based) Risk Assessment

No approach is currently available that allows us to state with certainty the real-world risks associated with exposure to any carcinogenic chemical at exposure concentrations other than those for which epidemiological data exist. Nevertheless, the uncertainties surrounding use of the LMS can be reduced if low-exposure extrapolation is based on mechanistic data, i.e., on the actual biological determinants of the carcinogenic response in animals. Use of mechanistic data does not have to be an all-or-none phenomenon. Even a partial description of the relevant mechanism(s) is better than none at all. Any mechanism-based model will be incomplete, inevitably omitting some critical aspects of the overall linkage between exposure and tumorigenic response. The short-range goal is only to incorporate available information with the expectation that the resulting risk prediction will be more accurate than a prediction ignoring available mechanistic data. A mechanism-based prediction will become more accurate as the description of mechanism is refined, providing an incentive for the design and conduct of mechanistic studies as a supplement to standard carcinogenicity bioassays. Of course, in the absence of adequate mechanistic data, the use of default procedures, including reliance on the LMS, may still be necessary.

Conolly et al. (15,16) described a generic, quantitative, mechanism-based, exposure–response model for chemical carcinogens. This proposed comprehensive model consists of a sequence of three submodels that collectively describe the overall process of chemical carcinogenesis. These submodels are tissue dosimetry, early tissue response, and cancer (late tissue response) (Fig. 1).

The tissue dosimetry submodel, usually based on a physiologically based pharmacokinetic (PBPK) model, is a mechanistic model for the pharmacokinetic behavior of a chemical and, more specifically, for the prediction of target tissue dosimetry. The essential features of PBPK models have been extensively discussed elsewhere (17–19).

The submodel for early tissue response describes, in as much mechanistic detail as possible, the quantitative relationship between the tissue dose of the chemical and its initial cellular effects, including DNA damage, cytolethality, or mitogenic stimulation. Any particular carcinogen may exert one or more of these effects, as well as others not specifically listed here. In general, much less work has been done on tissue response models than on PBPK models. Attempts to develop comprehensive, mechanism-based exposure–response models highlight the lack of progress in this area and can, thereby, help to focus research resources where they are most needed.

Finally, a mechanism-based cancer submodel, the Moolgavkar-Venzon-Knudson (MVK) model (20–22), describes, at the cellular level, the roles of cell replication and heritable, procarcinogenic genetic change (mutation) in the mechanism by which malignant tumors arise from a population of initially normal cells. Reasons for use of the MVK model in this context have been discussed elsewhere (15,16,23).
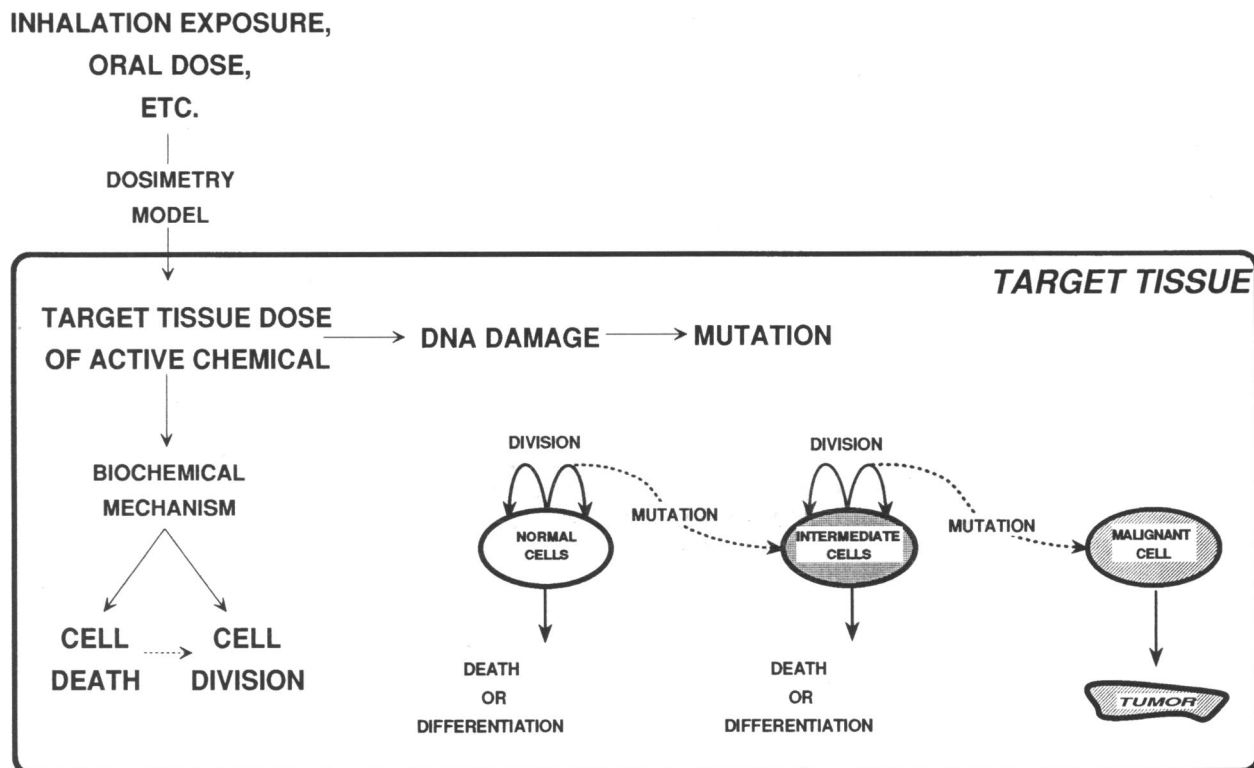
FIGURE 1. A generic, quantitative, mechanism-based exposure–response model for chemical carcinogens. A dosimetry model (often a physiologically based pharmacokinetic model) translates the exposure external to the organism to a dose of the active form of the chemical in the target tissue. Next, the mechanism linking the presence of the chemical in the tissue with early responses such as cytolethality, mitogenesis, or DNA damage is described in as much relevant detail as is available. Finally, the relationship between the early response and the mechanism by which normal cells become malignant, as described by the two-stage MVK model, is specified, again in as much relevant detail as is available.

As noted above, a key aspect of the overall exposure–response model as described by Conolly et al. (15,16) is the specification of an early tissue response model. This specification describes the linkage between the target tissue dose of carcinogen and the cell replication and mutation rate parameters of the MVK model (Fig. 1). For example, a cytolethal effect is described in the model as a temporary increase in the death rates of cells. Resulting regenerative cellular proliferation in the target tissue corresponds to an increase in cell division rates. Unrepaired DNA damage is correlated with an increase in the probability of procarcinogenic mutation per cell division. Thus, the early tissue response model specifies the biochemical mechanism of action of the carcinogen. Adequate, quantitative detail in this component of the overall exposure–response model is needed to identify behaviors that could substantially impact the shape of the overall exposure–tumor response curve. An exposure threshold for cytolethality, for example, would result in a corresponding exposure threshold for carcinogenic response as long as the chemical in question did not act simultaneously by some other, nonthreshold mechanism. Reitz et al. (24) have described a cancer risk assessment for chloroform that partially implements this generic approach.

The mechanism-based approach to cancer risk assessment will not necessarily provide lower estimates of risk relative to those obtained with the default methodology using the LMS model. Rather, when implemented properly, these approaches will generate estimated risk numbers that are more likely to correspond to the actual cancer risks which are, of course, determined by the biology of the exposed organism and the chemical/biochemical interactions of the carcinogen. Uncertainty in mechanism-based risk assessment is a consequence of measurement error, interindividual variation, and misspecification of mechanism (model). Each of these sources of uncertainty can be reduced through focused research and subsequent model refinement. A mechanism-based approach to risk assessment thus replaces the nebulous uncertainty associated with the default methodology (25) with much more clearly defined sources of uncertainty that can be reduced by relevant experiments.

Formaldehyde research has progressed to the point where data describing the biological mechanism linking formaldehyde exposure with tumor formation data are available (26–28). In the following section we consider how the default exposure–response assessment for formaldehyde can be improved by inclusion of these data.

Mechanism-based Risk Assessment for Formaldehyde

Tissue Dosimetry

Ongoing work at the Chemical Industry Institute of Toxicology (CIIT) is adapting the generic, mechanism-based exposure–dose–response model described above to cancer risk assessment for formaldehyde. The initial step in this direction was use of target tissue dosimetry data, rather than exposure concentration, for the exposure–dose–response characterization step of the overall risk assessment process. Even without specific consideration of mechanisms of tissue response, this innovation markedly affected the predictions of low-dose risk (9,29,30). Formaldehyde-derived DNA-protein cross-links (DPX) in the nasal respiratory epithelium of the F344 rat were used as a measure of delivered dose (29,30). Although these DPX may be causally linked to both formaldehyde-induced mutation and cytolethality, no such mechanistic role was assumed in using DPX as a target tissue dosimeter. The relationship between formaldehyde exposure concentration and DPX formation is highly nonlinear, with the rate of DPX formation increasing disproportionately as formaldehyde exposure levels increase (Fig. 2). The nonlinearity exists because some of the inhaled formaldehyde absorbed in the target region never exerts a toxic effect due to nonlinear clearance processes. For example, mucociliary function in the rat nose is inhibited by 15 ppm formaldehyde, but 6 ppm has much less effect, and 0.2 ppm has none at all (31).

The DPX data were used in place of exposure concentration as input to the LMS model and less risk was predicted for the lower exposure levels (Table 2). The potential impact of target tissue DPX on formaldehyde risk assessment has also recently been considered by the U.S. EPA (9). Their proposed revision of the 1987 formaldehyde risk assessment (11) predicts a lower risk at low levels of exposure when DPX data are used as input to the LMS in place of inhaled concentration of formaldehyde.
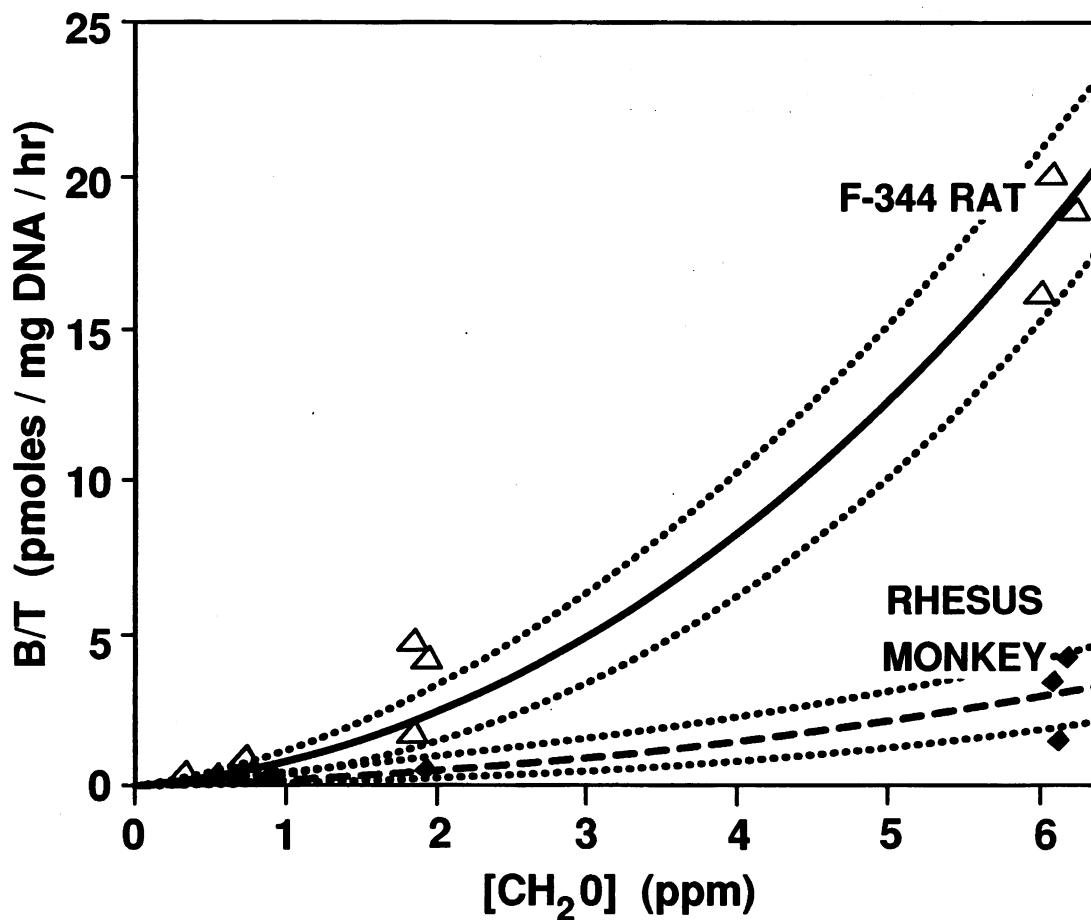

FIGURE 2. Average concentration of DNA-protein cross-links formed per unit time in the turbinates and lateral wall/septum of F344 rats and rhesus monkeys versus the airborne formaldehyde concentration. All animals were exposed for 6 hr to formaldehyde. Dashed lines are 95% confidence limits about the mean for each species. Modified from Casanova et al. (34).

Table 2. Upper 95% confidence-bound risk estimates per million, as derived with the three-stage carcinogenesis model.[a]

Formaldehyde concentration, ppm	Dose measure/species[b]		
	Air/rat	CVB/rat	CVB/monkey
1.0	1800	1200	140
0.5	810	420	41
0.1	160	74	8

[a]Modified from Starr (*30*).
[b]CVB: ^{14}C-formaldehyde covalent binding data, a measure of DNA–protein cross-links.

Target Tissue Response

In addition to target tissue dosimetry data, a large body of tissue response data has recently been obtained in a long-term pathogenesis study at CIIT (*23,26*) in which tissue responses were evaluated at several time points during chronic formaldehyde exposure at multiple concentrations. The essential features of this study were *a*) a large number of exposure levels (0, 2, 5, 10, and 15 ppm) *b*) 2 years of inhalation exposure with intermediate sacrifices at 1.5, 3, 6, 12, and 18 months, and *c*) collection of quantitative data on a large number of end points, including numbers of putative preneoplastic lesions and malignant tumors, numbers of cells in normal target tissue, preneoplastic lesions and tumors, and labeling indices for the estimation of cell division rates in normal tissue, preneoplastic lesions, and tumors. As already described (*23*),

this data set can be used to estimate all the parameters of a two-stage MVK model. The planned approach to estimating these parameter values and using the MVK model to develop risk estimates for formaldehyde has been described in detail (23) and is now reviewed briefly (Fig. 3).

First, the MVK model is configured for control animals (basal model). Estimates of target cell population size from the CIIT pathogenesis study, rates of cell division in control rats, and data or upper-bound estimates of numbers of preneoplastic lesions and malignant tumors are used to provide parameter values. These data are also used to estimate the baseline probabilities of procarcinogenic mutation per cell division (23) (Fig. 3). The basal model is modified by adding cell proliferation data from formaldehyde-exposed animals (replication model; Fig. 3). Finally, the replication model is modified by including a proportionality constant between DPX and an increase over the baseline value in the probability of procarcinogenic mutation per cell division (mutation model; Fig. 3). The value of the increase in mutation probability is estimated by fitting the model to actual tumor data (23).

For the basal, replication, and mutation MVK models, all parameters are based on laboratory data. This family of MVK models represents alternative, biologically based hypotheses about the mechanism of formaldehyde carcinogenesis in the F344 rat. The abilities of these alternative hypotheses, encoded as simulation models, to predict the actual tumor data are compared statistically (Table 3) (32).
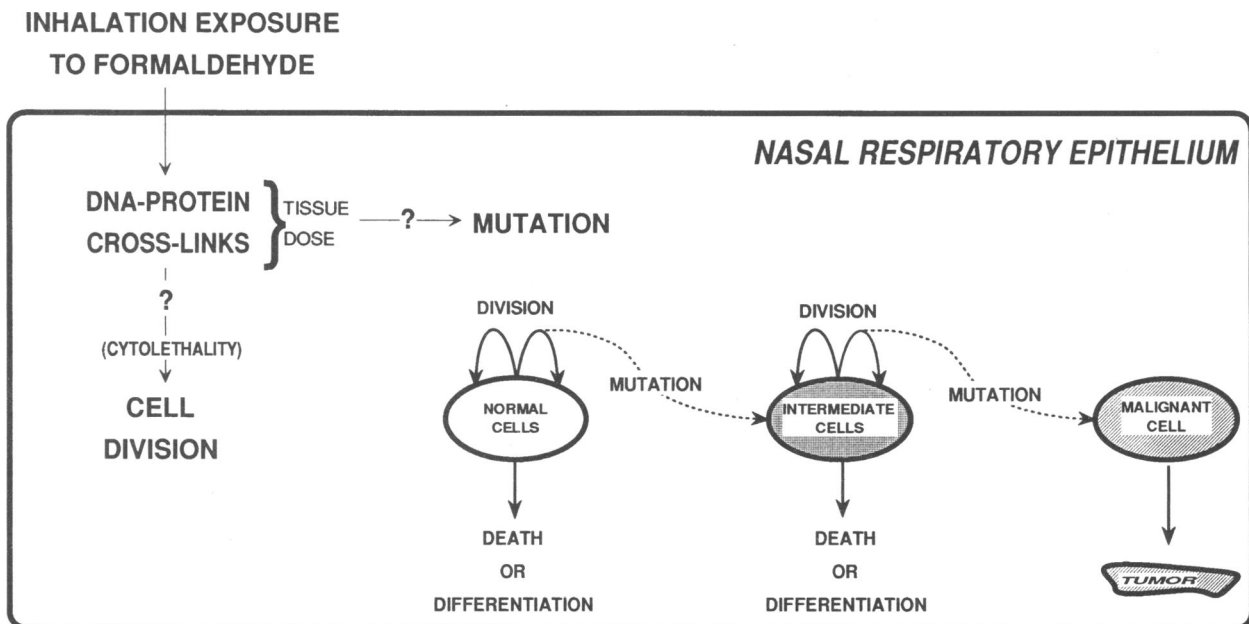
FIGURE 3. The generic exposure–dose–response model in Figure 1 modified for the nasal tumorigenicity of formaldehyde. Inhalation exposure to formaldehyde is correlated with DPX in the respiratory epithelium. DPX are used as a measure of tissue dose of reactive formaldehyde and have no other mechanistic significance is this description. The early consequences of the tissue dose, cytolethality, and ensuing cell replication, or DNA damage and mutation, are correlated with DPX. Cell replication data are used directly in the MVK model. A proportionality constant is defined between DPX and increases in the probabilities of mutation per cell division. The relative roles of cell replication and mutations in formaldehyde tumorigenesis are then evaluated as described in the text.

Table 3. Possible fits of formaldehyde cancer risk models to tumor data.

Case no.	Quality of fit[a]		
	Baseline model	Cell replication	Mutation and cell replication
1	10	10	10
2	1	10	10
3	1	1	10
4	1	5	10

[a]Units are arbitrary. Hypothetical best fit in each case is given a value of 10.

a) A significantly better simulation of the tumor data with the mutation model than with either the replication or basal models would indicate that a mutagenic effect of formaldehyde, in combination with stimulated cell replication, plays the major role in tumorigenesis. The hypothesis that formaldehyde is producing tumors simply by induction of mutation appears untenable in the face of the large increases in replication rates in affected tissues.

b) Equally good simulations of the tumor data by the mutation and replication models would suggest that formaldehyde-stimulated cell replication plays the major role in tumorigenesis as the fit to the tumor data is not, in this case, improved by describing a direct mutagenic effect.

c) Finally, if none of the models provide reasonable simulations of the tumor data, the hypothesis that the two-stage MVK description is appropriate for formaldehyde carcinogenesis will be reevaluated. This latter result would suggest that another model, perhaps with more than two stages, would be more appropriate. Development of more complex MVK-type models will then be pursued, while maintaining the approach of estimating as many of the parameter values as possible directly from data.

Animal to Human Scale-Up

Mechanism-based modeling of target tissue dosimetry and response, as described above, provides a description of the complete inhalation exposure–nasal tumor–response curve for the F344 rat. Human risk assessment requires consideration of how this description should be adjusted (scaled) to account for differences between rats and people. This scaling process includes interspecies adjustments for both tissue dosimetry and tissue response.

Scaling Tissue Dosimetry

Casanova et al. (33,34) described a pharmacokinetic model for DPX formation due to formaldehyde exposure that accurately reproduces measured DPX concentrations in both F344 rats and rhesus monkeys. This model has also been used to predict expected human DPX. A relatively straightforward scaling approach for tissue dosimetry is to use the model developed by Casanova and colleagues to predict human DPX for exposure scenarios of interest. Quantitative relationships established for the F344 rat

between DPX and stimulation of cell replication and DPX and an increase in the probability of mutation per cell division (see above) are then used to construct a formaldehyde risk model for humans. This human model would reflect scaling of target tissue dosimetry (DPX), but the use of the tissue response description developed for the rat.

Scaling Tissue Response

In the context of the present approach, interspecies scaling of target tissue response to formaldehyde reduces to scaling the parameters in the MVK model from rats to people (compare Figs. 1 and 3). People are much larger than rats and live many years longer. Other things being equal, people have more cells at risk for a longer time. Because control tumor rates are presumably similar in rats and humans, significant adjustments in the MVK description of cell replication rates and probabilities of mutation per cell division must be made to account for these interspecies differences. This is an area where we are badly in need of new data. Given the current lack of relevant data, it seems appropriate for the time being to simply use the tissue-response description defined for the rat as the basis for the human risk model.

Remaining Questions and Future Directions

The approach described here for developing a mechanism-based risk assessment model for formaldehyde carcinogenesis assumes that the rat is a good surrogate for the human. Epidemiological studies suggest that if formaldehyde causes any human cancers at all, they are most probably of the nasopharynx and lung (8,9). F344 rats exposed to formaldehyde have only developed tumors of the nasal respiratory epithelium (6). Thus, there may be significant differences in target site for formaldehyde carcinogenesis between rats and people. Here we consider how this issue can be addressed by extension of the mechanism-based risk model for formaldehyde.

With respect to tissue dosimetry and rat to human extrapolation, we are interested in how the regional dosimetry pattern in the entire rat respiratory tract, not just in the nose, compares to that for the human. Research underway at CIIT, and other laboratories, on computer simulation of airflow in the rat and human respiratory tracts has the potential, when coupled with physiochemical and mass flux equations, to provide accurate, quantitative predictions of regional deposition of inhaled gases in the nose, nasopharynx, and lung (35–37). Assuming equivalent responsiveness of the cells lining the airways in rats and people, knowledge of regional dosimetry, combined with the known regional tumor response in the rat, will permit prediction of the sites in the human respiratory tract most at risk for developing irritation and tumors in response to formaldehyde exposure. This approach allows prediction of the human exposure scenarios required to cause a measurable tumor incidence. Development of the

risk model to this point provides the opportunity for comparison of model predictions with epidemiological data.

These considerations of how simulation of regional tissue dosimetry may allow more sophisticated scaling of the mechanism-based formaldehyde risk model have all assumed equivalent tissue responsiveness. That is, we have assumed that the target tissue dose–carcinogenic response relationship for cells lining the respiratory tract is the same for rats and humans. This is almost certainly an oversimplification, but, for the present, there are no data with which to test the assumption. It is worth remembering that risk assessment, for formaldehyde or for any other chemical, is an evolving process. No risk assessment is likely, in the foreseeable future, to be both absolutely accurate and precise for every member of the heterogeneous human population. This is true even if the approach outlined here is modified to obtain risk estimate ranges, rather than point estimates, by using distributions of risk model parameter values to describe variability between people. Risk assessments will continue to evolve as our understanding of the physiological and biochemical mechanisms of tissue dosimetry and tissue response improves. Our approach for formaldehyde involves descriptions of dosimetry and response at the cellular and tissue levels. As we learn more, it should be possible to refine this approach also by describing the relevant carcinogenic mechanisms at the molecular level.

The mechanism-based approach described here is not specific to formaldehyde. The exposure-tissue dose–early tissue response-tumor response paradigm (Fig. 1) can be used as a starting point for mechanism-based assessment of carcinogenic risk for any chemical. It provides a framework for incorporation of mechanistic data as it is obtained not just when the mechanism has been exhaustively studied. The approach thus rewards mechanistic research designed to support human health risk assessment and uses the most current scientific understanding in protecting the health of the public from potentially adverse effects of toxic and carcinogenic chemicals.

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