EPA/600/R-08/103.
Analysis of the Sensitivity and Uncertainty in 2-stage Clonal Growth Models for Formaldehyde with Relevance to Other Biologically Based Dose Response (BBDR) Models.

The National Center for Environmental Assessment (NCEA) has published a series of papers addressing 2-stage clonal growth models for cancer as applied to formaldehyde. Herein, we summarize these papers, discuss the significance of this work for other BBDR applications, and provide journal reprints of two of these publications and a Web link to the scientific journal containing the third publication.

Introduction

As knowledge of the biology of cancer has evolved, researchers have sought to apply biologically motivated models to estimate risks from exposures to carcinogens. The understanding of carcinogenesis as a process with multiple steps, and the observed increasing patterns of cumulative cancer incidence with age, provided motivation for the multistage model of Armitage and Doll (1954). The terms in this model can be interpreted to represent a series of mutagenic changes or more general distinct stages related to carcinogenesis. In turn, this model led to the development of the linearized multistage model that the U.S. EPA has employed in many assessments to extrapolate risks for environmental exposure to carcinogens (Crump et al., 1976; U.S. EPA, 1986). Clonal growth models, on the other hand, represent carcinogenesis as a process that involves initial and subsequent mutations, with growth and focal expansion of cells subsequent to mutations (Moolgavkar and Venzon, 1979; Moolgavkar and Knudson, 1981; Portier and Kopp-Schneider, 1991; Portier et al., 1996). Clonal growth models are valuable due to their ability to represent the different effects that carcinogens may exert on rates of mutation or stimulation of cellular growth. As such, clonal growth models also represent important examples of biologically motivated models, which foster descriptions of multiple events in complex disease processes.

Clonal growth modeling efforts have been fruitful in generating hypotheses, leading to a better understanding of the biology and the implications for human health risk. Inferences from these models have also highlighted relevant data gaps. Examples of such chemical carcinogens include diesel exhaust emissions, dioxin, and trichloroethylene. Chen and Oberdorster (1996) successfully linked pharmacokinetic and pharmacodynamic information in an integrated lung dosimetry and clonal growth dose-response model for diesel exhaust using rodent bioassay data. The modeling allowed them to study the relative roles of the particulate and adsorbed volatile organic components (e.g., polycyclic aromatic hydrocarbons) of diesel in the cancer process at various exposures. Their modeling results suggest that lung tumors observed in the rat bioassays may arise mainly due to the particulate effects, while the mutagenic and

genotoxic effects from particle-associated organics may play a primary role in tumorigenesis at low doses relevant to most human exposures. Chen (2000) used clonal growth modeling in a similar vein for modeling liver cancer risk upon exposure to trichloroethylene (TCE) based upon the extensive bioassay data for this compound and its two metabolites, dichloroacetic acid (DCA) and trichloroacetic acid (TCA). Their modeling results indicate that the effects of DCA, alone, could potentially account for TCE-induced liver tumorigenicity in mice. The authors did not characterize their effort as enabling a more accurate estimate of low-dose risk but, rather, as elucidating the effect of plausible biological assumptions on risk.

Several laboratories have contributed to the clonal growth modeling of the cancer and non-cancer effects of dioxin, addressing several important questions (e.g., Moolgavkar et al., 1996; Portier and Kohn, 1996; Portier et al., 1996; Conolly and Andersen, 1997; Portier, 2000; Luebeck et al., 2000; Luebeck et al., 1995). For example, while dioxin is generally not considered a mutagen as per *in vitro* studies, clonal growth modeling efforts suggest that dioxin-induced secondary mechanisms associated with mutations could be important factors in the carcinogenicity of this chemical (Portier et al.,1996; Moolgavkar et al., 1996). Additionally, Portier and co-workers conclude that the data do not fully support a threshold in the cancer dose-response for dioxin (Portier, 2000). In a different contribution to the debate, the clonal growth modeling of Conolly and Andersen (1997) proposes a U-shaped dose-response curve for the number of altered foci per unit volume. The different approaches taken indicate how modeling investigations can underscore significant biological uncertainties affecting dose-response assessments.

These clonal growth and other BBDR modeling efforts typically require considerable effort—both in gathering the relevant empirical data and in computational resources. Notably, however, the clonal growth models in the literature (and sophisticated BBDR models more broadly) have generally <u>not</u> been used in formal risk assessment to predict risk at human exposures from toxicological data. A prominent exception in this regard is the formaldehyde modeling effort by scientists at the Hamner Institutes for Health Sciences (formerly CIIT) in which a clonal growth model and associated dosimetry calculations were developed specifically for use in extrapolation of cancer risk.

In a series of papers and a health risk assessment report, scientists at the CIIT Hamner Institutes developed a model (the “CIIT model”) for estimating respiratory cancer risk due to inhaled formaldehyde, within a conceptual framework, that incorporates substantial mechanistic information and advanced computational methods at both the toxicokinetic and toxicodynamic levels.

The remainder of our report summarizes published *NCEA* investigation of the mathematical and biological assumptions of the CIIT model and the characterization of associated uncertainty in risk predictions (Subramaniam et al.,

2008; Subramaniam et al., 2007; Crump et al., 2008). The presence of considerable biological data at various levels and advances in computational resources have made it possible to examine uncertainties somewhat more extensively than in the past.

We used two general questions to frame our approach regarding the application of models that seek to increase application of biological data in risk assessment:

(1) To what extent does the formulation of the model allow sound characterization (and hopefully reduction) of uncertainties present in cancer risk assessment?
(2) To what extent does the modeling approach allow characterization of the relative weights of key events in the mode-of-action of a carcinogen?

NCEA's research regarding the CIIT model showcases an important examination of these questions. In addition to strengthening the characterization of formaldehyde risks, NCEA's work can provide insights for investigators looking towards future applications of BBDR models in risk assessment.

Synopsis of NCEA publications

Subramaniam et al. (2008) reviews key biological and statistical uncertainties that need careful evaluation if such two-stage clonal expansion models are to be used for extrapolation of cancer risk from animal bioassays to human exposure. Broadly, these pertain to the following issues:

- The sensitivity of the dose response to constraints on the heterogeneity of historical control animals
- The use and interpretation of experimental labeling index and tumor data, and the uncertainty and variability in these data
- The evaluation and biological interpretation of the estimated parameters
- The uncertainties in model specification—in particular that of initiated cells. Given the paucity of data on the kinetics of initiated cells, Subramaniam et al. (2008) explores various biological inferences that were indicated by the CIIT formaldehyde modeling and examines their plausibility in the face of known biology.

Subramaniam et al. (2008) also identifies key uncertainties in the scale-up of the CIIT model to humans, focusing on assumptions that underlie the model parameters for cell replication rates and formaldehyde-induced mutation. The authors discuss uncertainties in identifying parameter values in the model used to estimate and extrapolate DNA protein cross-link levels.

Subramaniam et al. (2007) implements a quantitative analysis of select uncertainties in the CIIT model for rats. This paper implements solutions to the 2-stage cancer model that are mathematically valid for non-homogeneous models (i.e., models with time-dependent parameters), thus, accounting for time

dependence in variables. The original CIIT model used a solution method that was not valid for time-dependent parameters. In this re-implementation, the authors examine the sensitivity of model predictions to pooling historical and concurrent control data and to lumping sacrificed animals, in which tumors were discovered incidentally with those in which death was caused by the tumors. An inference of the CIIT modeling approach is that formaldehyde-induced tumorigenicity could be optimally explained without the role of formaldehyde's mutagenic action. Subramaniam et al. (2007) examines the strength of this result. The primary conclusions are as follows:

- CIIT model results are not significantly altered with the non-homogeneous solutions.
- Dose-response predictions below the range of exposures where tumors occurred in the bioassays are highly sensitive to the choice of control data.
- In the range of exposures where tumors were observed, the model attributes up to 74% of the added tumor probability to formaldehyde's mutagenic action when the reanalysis restricted the use of the National Toxicology Program (NTP) historical control data to only those obtained from inhalation exposures.
- Model results are insensitive to hourly or daily temporal variations in DNA protein cross-link (DPX) concentration, a surrogate for the dose-metric linked to formaldehyde-induced mutations, prompting these authors to utilize weekly averages for this quantity.

Various other biological and mathematical uncertainties in the model identified (qualitatively) in Subramaniam et al. (2008) have been retained unmodified in this analysis. These include the model specification of initiated cell division and death rates, and the uncertainty and variability in the dose-response for cell replication rates.

Crump et al. (2008), the third paper in this series, evaluates the modeling in Conolly et al. (2004). In this model, risk estimated using the rat model in Conolly et al. (2003) was extrapolated to human exposures in Conolly et al. (2004). The primary result of the human model is that the risks associated with inhaled formaldehyde are *de-minimis* at relevant human exposure levels. Crump et al. (2008) presents a limited sensitivity analysis of the formaldehyde human model by examining the impact of two key factors only while keeping all other major uncertainties unchanged:

- The effect upon the human model of which controls are used in the animal model
- The impact of the lack of data on the division rates and death rates of initiated cells

On both these accounts, analysis in Crump et al. (2008) shows the estimates of human risk in Conolly et al. (2004) to be hyper-sensitive to their modeling assumptions:

- When the control animals from the National Toxicology Program (NTP) studies are replaced with control animals only from NTP inhalation studies, estimates of human risk are increased by 50-fold. When only concurrent control rats are used, the model does not provide any upper bound to human risk.
- In Crump et al. (2008), a decrease in initiated cell replication rates at low doses, as seen in the J-shaped curve used in Conolly et al. (2003), is retained, but varied to a small degree. The exercise shows that with very small numerical perturbations one could obtain quantitative risks in the human model ranging from negative up to 4 orders of magnitude higher than the "conservative estimates" calculated by Conolly et al. (2003). These modifications are just as consistent with the underlying data used to construct the model and fit the bioassay data as well as the original model that was based upon the Conolly et al. (2003) assumptions.

Summary and implications

The results developed in the three papers discussed above provide a significant reference point for other potential applications of BBDR modeling in quantitative health risk assessment. Biologically motivated models that explicitly incorporate mechanistic information have the potential to provide improved technical tools for human health risk assessment and to support a more scientifically based evaluation of uncertainty in health risk predictions. The realization of this potential depends on adequate characterization of the impacts of model assumptions on bottom-line risk predictions. BBDR modeling can make the resulting uncertainties explicit and identifiable. Nonetheless, the usefulness of BBDR models over standard statistical modeling approaches for risk estimation at human exposures is not evident *a priori*.

The wide range of plausible dose-response estimates that were obtained in NCEA's work show that clonal growth modeling, in this case, does not serve to usefully narrow uncertainty in the range of low-dose human risk for this compound. While some model realizations would predict very low risks at the low dose (or even reduction in baseline risks), other realizations can predict risks as high as (or substantially higher than) those predicted by U.S. EPA's baseline methods of low-dose linear risk assessment (U.S. EPA, 2005). Thus, the analyses published in these papers do not support the claim in Conolly et al. (2004) that their risk estimate represents a conservative estimate on human risk in the face of model uncertainties.

The uncertainty in the Conolly et al. (2003, 2004) models is particularly acute because there are no data on the formation or the growth rates of initiated cells due to formaldehyde exposure. These authors made assumptions about division rates of initiated cells based upon analogy with data on normal cells, and evaluated these assumptions by comparing model predictions on tumor rates with animal tumor data. However, as concluded in Crump et al. (2008), the

changes made to the assumptions regarding initiated cells are numerically so small that it would appear to be virtually impossible to obtain sufficiently precise measurements of cell replication rates to rule out the model variations considered in this exercise. The hypersensitivity of clonal growth modeling to these uncertainties suggests that other attempts to quantify low-dose human risk using animal data on intermediate upstream steps in the carcinogenic process may encounter related uncertainties, even when substantial biological data are available.

Modeling assumptions and uncertainties pertaining to upstream intermediate steps in biologically motivated models are generally significant and can be strongly amplified when propagated downstream to risk end points. In essence, the BBDR process involves replacing *general relationships*, having some empirical support in a baseline (or "default") approach to risk assessment, with much *more specific relationships and assumptions*. These specific assumptions, while presumably appearing scientifically plausible, may have limited empirical support. In addition, the complexity of underlying parameters and their relationship to the empirical measurements can often lead to loss of transparency, a key feature for consistent regulatory utility (OMB, 2006). In the case of statistical model fits to data on frank toxicological effects, different model forms typically produce widely different risk estimates outside the observed data (NRC, 1983). NCEA's experience with formaldehyde modeling indicates that similar uncertainties can occur with biologically based models. In biologically based models, the statistical uncertainty in the dose response for tumor (or other effect) incidence is replaced by the statistical uncertainty propagated from fitting models to some intermediate upstream step. It is an open question whether (and under what circumstances) overall statistical uncertainty in extrapolating tumor risk can be reduced by this transfer of uncertainty.

A second question pertains to the extent to which modeling approaches can be used to deliberate upon the relative weights of key events in the mode-of-action of a putative carcinogen. The work presented in the papers in this summary shows that the model-based conclusion in Conolly et al. (2003), that formaldehyde's direct mutagenic action is not relevant to its tumorigenicity, is highly sensitive to the specific data that was utilized. It was shown that the clonal growth modeling could also substantiate the opposite point of view—that formaldehyde induced mutations played a key role in carcinogenesis. Thus, the analyses presented here emphasize that uncertainty and sensitivity analyses are essential tools when evaluating inferences about fundamental biological processes (e.g., modes of action) that may be drawn from a BBDR model.

NCEA's work in examining properties of formaldehyde clonal growth modeling also illustrates the complexities and substantial resource requirements involved in evaluating BBDR models. In the case of complex models, such as the CIIT formaldehyde model, even a basic sensitivity analyses can quickly proliferate into a large number of scenarios that need to be examined.

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