Letter to the Editor

Formaldehyde Risk Assessment

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We would like to comment on the paper by Crump et al. (2008), ‘Sensitivity analysis of biologically motivated model for formaldehyde-induced respiratory cancer in humans’. We are authors of the formaldehyde cancer risk assessment described in Conolly et al. (2003, 2004) that is the subject of the Crump et al. paper. The focus of our comments is not on cancer risks per se associated with exposure to formaldehyde but, rather, on technical issues that arise in development of biologically motivated computational models (BMM) such as that described by Conolly et al. (2003, 2004). Indeed, our comments are for the most part generically relevant to the development of these kinds of models.

A two-stage clonal growth (CG) model is an important component of the suite of computational models that were developed in support of the assessment by Conolly et al. In the following, we provide an overview of biologically motivated computational modeling (Overview of biologically motivated modeling) and then proceed to focus largely on two aspects of Crump et al. These are (i) their alteration of sensitive parameters of the CG model (Crump et al. reparameterization of the CG model) and (ii) their implicit suggestion that CG modeling is inappropriate if data on the intermediate cell compartment of the model are incomplete, even when the model is supported by an otherwise rich dataset (The value of experimental characterization of mechanisms of dose–response). We also provide a limited commentary on the issue of control groups, a topic for which we think that Crump et al. have made a useful contribution in this ongoing consideration of dose–response modeling for the carcinogenicity of formaldehyde (Control groups).

OVERVIEW OF BIOLOGICALLY MOTIVATED MODELING

The computational models that comprise the assessment by Conolly et al. are, as noted in Crump et al., biologically motivated. The purpose of a BMM is to gain insight into a biological behavior or topic of interest—in this case, the development of cancer in rats and people exposed to formaldehyde—by explicitly representing the biological mechanism or mechanisms that link exposure with carcinogenic effect. It is reasonable to consider a BMM as representing an explicit hypothesis that characterizes the mechanisms. To the extent possible, the BMM is specified at a level of biological detail consistent with available data. The number of parameters that must be estimated by formal optimization is therefore minimized, though it is seldom if ever possible to eliminate the need for some amount of statistical optimization.

Given the complexity of biological systems and the need to minimize the number of parameters estimated by formal optimization, it is important to use Occam’s Razor during model development. Also known as the law of parsimony, Occam’s Razor states that ‘one should not increase, beyond what is necessary, the number of entities required to explain anything.’ In practice, only one or at most a limited number of alternative models are possible when Occam’s Razor is used, while an infinite number of alternative models are possible when it is not. (For the sake of added perspective, we note that the characteristics of BMMs described in this section differentiate them from statistical data fits of dose–response, such as the linearized multistage and benchmark dose models.)

Consistency with known behaviors of the biological system (e.g. a human, a rat or even an in vitro system) that a BMM describes is an important criterion in the assessment of model validity. If the behavior of a BMM extends outside the range of known biological behavior, then the structure and parameterization of the model must be seriously questioned.

CRUMP ET AL. REPARAMETERIZATION OF THE CG MODEL

As part of their sensitivity analysis, Crump et al. develop a new and more complicated version of the CG model. This new version predicts unrealistically high lifetime cancer risk for both ambient air and industrial workplace exposures to formaldehyde (see Figs 3 and 8 in their paper). Specifically, the new
version of the formaldehyde risk model developed by Crump et al. introduces arbitrary adjustments to the kinetics of intermediate (I) cells in the CG model (see Figs 4 and 5 in their paper). The prediction of greater risk is only possible, if consistency with the bioassay tumor data is to be maintained, by limiting these adjustments to low levels of exposure to formaldehyde. This new model predicts lifetime cancer risk for formaldehyde exposure of 0.01 on up to essentially 1 (everyone gets cancer) for exposures on the order of 0.1 p.p.m. Numerous epidemiological studies have been conducted where 0.1 p.p.m. or much higher levels of exposure to formaldehyde occurred. No study has ever indicated risk levels anything like those predicted by the Crump et al. modified versions of the Conolly et al. assessment. Crump et al. indirectly address this point. They say:

We have not examined whether the upper end of the range of additional risk is consistent with existing human epidemiology because that is not germane to the point we are making.

We strongly disagree with their assertion that prediction of risks that are inconsistent with existing human epidemiology is not germane. The unrealistically high risks predicted by Crump et al. tell us that the parameter adjustments they make are inappropriate. The sensitivity analysis of any BMM should be constrained by respect for the biological realism of model-predicted behaviors. The Crump et al. analysis has in fact identified a boundary for the reasonable adjustment of I cell growth kinetics in response to formaldehyde exposure.

Crump et al. refer to the work of Heck and Casanova (1999) to justify their novel treatment of I cells but in their justification Crump et al. misunderstand Heck and Casanova. The latter posited that DNA–protein cross-links (DPX) formed by inhaled formaldehyde physically block the replication complex as it moves along the DNA strand during the S phase of the cell cycle. This physical blockage would slow the overall rate of cell division, as the replication complex would remain stalled until the offending DPX was removed. Crump et al., in referencing Heck and Casanova, incorrectly state that formation of DPX activates a checkpoint in the cell cycle, providing extra time for repair of the DNA lesion. They then say that if the mutation that creates an I cell from a normal cell impairs the ability to activate the checkpoint, then I cells would divide more rapidly than normal cells. Linkage of their argument to Heck and Casanova is spurious, however, since Heck and Casanova (1999) did not posit a role for cell cycle checkpoints in their explanation of how DPX affect the rate of cell division.

We also note that the treatment by Crump et al. of I cell kinetics involves the dissociation of I cell kinetics from the kinetics of normal cells, specifically at low, non-tumorigenic levels of exposure to formaldehyde. This is a significant departure from the treatment of I cells in the assessment by Conolly et al., where I cell kinetics differ from but are linked with the kinetics of normal cells for all conditions of exposure to formaldehyde. I cells do not grow in an unconstrained manner (Dragan et al., 1996; Mehta, 2007), as do truly carcinogenic cells (otherwise I cells would be cancer cells), and this constraint is observed by Conolly et al., where the effects of formaldehyde on normal cell kinetics also affect I cells.

In Conolly et al., the relationship between I cell kinetics and those of normal cells is defined by two adjustable parameters. One, called multb, specifies a fixed growth advantage for I cells relative to N cells while the other, called multfc, adjusts this growth advantage as a continuous function of the dose of formaldehyde. Use of only multb provides a good description of the rat tumor data, but with a slightly diminished ability to simultaneously fit the tumor data at 10 and 15 p.p.m. Use of multb and multfc together is, we think, a reasonable implementation of Occam’s Razor, given the lack of I cell-specific data, though one could argue that use of only multb might have been even more consistent with the Razor.

Although they do not say so in their paper, the novel behaviors for I cells posited by Crump et al. would require a low-dose mitogenic mechanism for formaldehyde that is specific to I cells and that does not occur in normal cells. In fact, the data for normal cells show a low dose decrease in division rate (see Fig. 6 in their paper), quite the opposite of mitotic index when cells in culture were treated with 0.1 mM formaldehyde, a non-cytotoxic concentration, but not at higher concentrations. In relying on Tyihak et al., however, Crump et al. are ignoring a vast body of well-established literature showing that, in vivo, the growth kinetics of cells are tightly regulated by intercellular communication processes that are missing or are disrupted in vitro (e.g. Mehta, 2007). There is little reason to expect that the data of Tyihak et al. are relevant to the assessment by Conolly et al., which is based on a large body of data collected in vivo.
To summarize, the prediction by Crump et al. of much greater risk due to an increased rate of division of I cells at low levels of exposure to formaldehyde depends on (i) a speculative treatment of I cell kinetics that has neither empirical nor theoretical support and (ii) a low-dose effect of formaldehyde on cell kinetics justified by reference to \textit{in vitro} data of questionable relevance. Moreover, in the absence of any supporting data, the complex description of I cell kinetics introduced by Crump et al. is not consistent with use of Occam’s Razor.

**THE VALUE OF EXPERIMENTAL CHARACTERIZATION OF MECHANISMS OF DOSE–RESPONSE**

In the discussion of their paper, Crump et al. say:

Thus, the previous paragraphs suggest that the changes made in our analysis to the assumption by Conolly et al. regarding the dose response for the division rate of initiated cells are not implausible. Nevertheless, given that these changes are very small, and that there are no data on initiated cells, we believe that before the Conolly et al. model is accepted as conservative, the onus should be on showing that these small differences cannot occur.

We interpret this passage as saying that, with regard to I cells, one should obtain sufficiently extensive data to characterize their dose–response behaviors with minimal residual uncertainty. Implicitly then, in the absence of such data, one should not proceed with a cancer risk assessment using a CG model. We disagree. Formaldehyde is unusual in that (i) the bioassays collected both time–course and dose–response tumor data, (ii) extensive mechanistic studies were conducted that identified key event precursors to the tumor response and (iii) sophisticated pharmacokinetic models are available for rats and humans. It can be argued that, with respect to the exposure–tumor response continuum, the only significant data gap is that for I cells. Do we not use all these data in an integrated manner, as was done by Conolly et al. (2003, 2004), just because data on I cells are lacking? When a straightforward parameterization of I cells was developed, consistent with biological principles and with Occam’s Razor, as discussed above, optimal values of the I cell parameters were readily identifiable. The only meaningful debate here, in our opinion, is over what is reasonable biologically and over the appropriate use of Occam’s Razor. As previously discussed, we maintain that our implementation is consistent with biology and with the Razor, while that of Crump et al. is not.

**CONTROL GROUPS**

The most useful contribution provided by the Crump paper is their consideration of alternative control groups for the CG modeling, in particular the recognition that the spontaneous tumor rate in inhalation-only control is statistically significantly different from that of the gavage controls. However, Crump et al., in their consideration of the use of the concurrent control data, make the following statement in the abstract of their paper concerning predicted risk:

When only concurrent control rats are used, the model does not provide any upper bound (UB) to human risk.

The model described by Conolly et al. (2003, 2004) does not consider how variability in spontaneous tumor incidence in control groups affects predicted risks and, moreover, Conolly et al. (2004) clearly state that the predicted risks are not upper bounds. If we had considered the issue of control group variability, we would not have reached conclusions that are at variance with biological reality. As discussed elsewhere in this letter, epidemiological studies have shown that exposure to formaldehyde does not result in very large cancer risks, risks that approach something in the range of 0.01–1. We would have carefully examined the epidemiological literature to estimate upper bound constraints on the relevant parameters to ensure that the model predictions made sense. We thus think that the statement by Crump et al., to the degree that it is directed toward the work of Conolly et al. (2003, 2004) rather than toward their own modifications of that work, is gratuitous.

In summary, we have two main concerns with the conclusions of Crump et al. First, they inappropriately adjust the parameterization of I cells in the CG model and obtain correspondingly inappropriate estimates of cancer risk. Second, they imply that CG modeling should not be used when data for I cells are lacking, even though the rest of the relevant database is richly populated. We think that this position inappropriately devalues the many years of research that have illuminated the mechanistic basis for the tumors observed in rats.

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REFERENCES


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