Ann. Occup. Hyg., Vol. 52, No. 6, pp. 481–495, 2008

doi:10.1093/annhyg/men038

Sensitivity Analysis of Biologically Motivated Model for Formaldehyde-Induced Respiratory Cancer in Humans

KENNY S. CRUMP[1,3]*, CHAO CHEN[2], JOHN F. FOX[2], CYNTHIA VAN LANDINGHAM[1] and RAVI SUBRAMANIAM[2]

[1]*ENVIRON International Corporation, 1900 North, 18th Street, Suite 804, Monroe, LA 71201, USA;*
[2]*National Center for Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Mail Code 8623D, 1200 Pennsylvania Avenue, NW, Washington, DC 20460, USA*

Received 2 April 2008; in final form 30 May 2008; published online 14 July 2008

Conolly *et al.* (2003, 2004) developed biologically motivated models of formaldehyde carcinogenicity in F344 rats and humans based on a two-stage clonal expansion model of cancer. Based on the human model, Conolly *et al.* (2004) claimed that cancer risks associated with inhaled formaldehyde are deminimis at relevant human exposure levels. However, they did not conduct a sensitivity analysis to evaluate the robustness of this conclusion. Here, we present a limited sensitivity analysis of the formaldehyde human model. We show that 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 (UB) to human risk. No data went into the model on the effect of formaldehyde on the division rates and death rates of initiated cells. We show that slight numerical perturbations to the Conolly *et al.* assumptions regarding these rates can be made that are equally consistent with the underlying data used to construct the model, but produce estimates of human risk ranging anywhere from negative up to 10 000 times higher than those deemed by Conolly *et al.* to be 'conservative'. Thus, we conclude that estimates of human risk by Conolly *et al.* (2004) are extremely sensitive to modeling assumptions. This calls into question the basis for the Conolly *et al.* claim of de minimis human risk and suggests caution in using the model to derive human exposure standards for formaldehyde.

Keywords: formaldehyde; nasal cancer; quantitative risk assessment; respiratory cancer; sensitivity analysis; two-stage clonal expansion model

INTRODUCTION

Inhaled formaldehyde induces squamous cell carcinomas (SCCs) in rat nasal tissue with a very steep dose response (Kerns *et al.*, 1983; Monticello *et al.*, 1996). The time-to-tumor data from these studies and results from a number of other investigations (Casanova *et al.*, 1991; Monticello *et al.*, 1991; Casanova *et al.*, 1994; Subramaniam *et al.*, 1998; Conolly *et al.*, 2000; Kimbell *et al.*, 2001a,b; Overton *et al.*, 2001) were used to develop biologically motivated models for formaldehyde carcinogenicity in F344 rats (Conolly *et al.*, 2003) and humans (Conolly *et al.*, 2004). These models are based on a two-stage clonal expansion model of cancer (Moolgavkar and Venzon, 1979; Moolgavkar and Knudson, 1981; Moolgavkar *et al.*, 1988).

The formaldehyde rat model accounts for two modes of action that may be relevant to formaldehyde carcinogenicity. First, the model incorporates an indirect mode of action in which the regenerative cell proliferation in response to formaldehyde cytotoxicity increases the probability of errors in DNA replication. This mode of action is modeled using

*Author to whom correspondence should be addressed.
Tel: +318-257-4051; fax: +318-257-3182;
e-mail: kennycrump@email.com
[3]Present address: Department of Mathematics and Statistics, Louisiana Tech University, Railroad Avenue, George T. Madison Hall, Room 330, PO Box 10348, Ruston, LA 71272-0046, USA.
The original version was incorrect. A few errors had not been corrected.

labeling data on normal cells in nasal mucosa of rats exposed to formaldehyde. Second, there is a possible direct mutagenic mode of action, based on information indicating that formaldehyde is mutagenic (Grafstrom *et al.*, 1985; Heck *et al.*, 1990; Speit and Merk, 2002) and which is modeled using rat data on formaldehyde production of DNA–protein cross-links (DPXs) (Casanova *et al.*, 1994; Conolly *et al.*, 2000). An important and novel feature of the two-stage modeling in Conolly *et al.* (2003) is that DPXs and cell replication and death rates are linked to regional formaldehyde flux simulated using computational fluid dynamics (CFDs) modeling (Subramaniam *et al.*, 1998).

No data are available on initiated cells, and consequently two critical assumptions had to be made: (i) The effect of formaldehyde upon the division rates for these cells is modeled assuming a two-parameter function that relates these unknown rates to those of normal cells. (ii) Death rates of initiated cells are assumed to equal the division rates of normal cells for all values of formaldehyde flux. The rat model involves six statistical parameters that are estimated by fitting the model to the rat formaldehyde bioassay data, plus data from several thousand control rats from bioassays conducted by the National Toxicology Program (NTP). The resulting model predicts the probability of a nasal SCC in the F344 rat as a function of age and exposure to formaldehyde.

The human model for formaldehyde carcinogenicity (Conolly *et al.*, 2004) is conceptually very similar to the rat model; however, the model does not incorporate any data on human responses to formaldehyde exposure. Rates of cell division and cell death are, with a minor modification, assumed to be the same in humans as in rats. The concentration of formaldehyde-induced DPX in humans is estimated by 'scaling up' from values obtained from experiments in the F344 rat and rhesus monkey. The statistical parameters for the human model are either estimated by fitting the model to the human background data, assumed to have the same value as obtained in the rat model, or (in one case) fixed at a value suggested by the epidemiological literature.

Based upon weighting rat cell labeling data in Monticello *et al.* (1991, 1996) by exposure time and averaging over six nasal sites, the response of cell replication to formaldehyde is assumed to either have a 'J shape', in which at low exposures the replication rates are below background, or a 'hockey stick' shape, in which there is a threshold of exposure below which formaldehyde has no effect. The formaldehyde-induced probability of mutation per cell generation (added to the spontaneous mutational probability) in the Conolly *et al.* models was assumed to be proportional to the DPX concentration in the tissue. In the development of the human model, Conolly *et al.* (2004) made what they considered to be conservative estimates of human risk by use of the hockey stick model, use of overall respiratory tract cancer incidence data in humans and evaluating the model at the statistical UB of the proportionality parameter relating DPX to the probability of mutation. Based on the estimates of additional human risk obtained in this fashion, Conolly *et al.* (2004) concluded that 'this analysis of the human implications of the rat SCC data indicates that (1) cancer risks associated with inhaled formaldehyde are *de minimis* (10^{-6} or less) at relevant human exposure levels and (2) protection from the non-cancer effects of formaldehyde should be sufficient to protect from its potential carcinogenic effects'. However, Conolly *et al.* did not conduct a sensitivity analysis of their model to determine the extent to which changes to their assumptions might also be consistent with their data but which produce higher estimates of human risk.

The results in Conolly *et al.* (2004) have been used by regulatory agencies to support exposure standards for formaldehyde (Health Canada, 2001; Liteplo and Meek, 2003; BfR (Germany), 2006; the German MAK Commission, 2006; USEPA, 2006a,b). The attractive features of this model include its incorporation of biological knowledge regarding the mechanisms of formaldehyde carcinogenicity and incorporation of a substantial amount of biological data in the quantitative modeling of these mechanisms. However, to more fully characterize the model and determine the appropriate use of modeling results for regulatory purposes, it is informative to conduct a sensitivity analysis of the model to determine how robust the model predictions are and to see if other biologically plausible assumptions might also be consistent with the available data but predict different estimates of human risk.

This paper presents the results of a limited sensitivity analysis of the Conolly *et al.* (2004) human model. This analysis is limited to evaluating the effect upon the human model of (i) the use of the alternative sets of control data for the rat bioassay data that were considered in the sensitivity analysis of the rat model (Subramaniam *et al.*, 2007) and (ii) minor perturbations in model assumptions regarding the effect of formaldehyde upon the division rates of initiated cells. There are no data to even crudely inform the kinetics of initiated cells for use in the models, even in rats, and the two-stage clonal expansion model is very sensitive to initiated cell kinetics (Crump, 1994a,b; Gaylor and Zheng, 1996). We examine this sensitivity in the formaldehyde model while constraining variations in model assumptions to only those that do not meaningfully degrade the fit of the model to the underlying data. Our results are compared to those of Conolly *et al.* (2004) with respect to concordance with available data and predicted levels of additional human risk associated with formaldehyde exposure.

Our sensitivity analysis of the Conolly *et al.* model involves, as one would expect, making certain modifications to this model and presenting consequences of these modifications. However, we wish to make it abundantly clear that we are not proposing these modifications as alternatives to the Conolly *et al.* model for assessing human risk from exposure to formaldehyde. We present results from these modifications only to provide insights on the sensitivity of the Conolly *et al.* model to certain assumptions in the model.

METHODS

Description of the Conolly et al. (2003, 2004) formaldehyde models

The Conolly *et al.* (2004) human model for formaldehyde carcinogenicity is patterned closely after the rat model (Conolly *et al.*, 2003) and uses much of the same data. We briefly describe these models below and refer the reader to earlier papers (Conolly *et al.*, 2003, 2004; Subramaniam *et al.*, 2007) and to supplementary material (available at *Annals of Occupational Hygiene* online) for further modeling and statistical details.

The two-stage clonal expansion model, upon which both the Conolly *et al.* (2003, 2004) formaldehyde models are based, is a stochastic Markov birth and death process that is defined in terms of the following parameters: N, number of normal cells that are eligible for progression to malignancy; α_N, division rate of normal cells (h^{-1}); μ_N, rate at which an initiated cell is formed by mutation of a normal cell (per cell division of a normal cell); α_I, division rate of an initiated cell (h^{-1}); β_I, death rate of an initiated cell (h^{-1}); μ_I, rate at which a malignant cell is formed by mutation of an initiated cell (per cell division of an initiated cell) and D, delay from occurrence of the first malignant cell to the resulting death of the host.

Some of these parameters in Conolly *et al.* (2003) incorporate data from auxiliary studies of cell replication (α_N, α_I and β_I) or DPX formation (μ_N and μ_I). However, all of them except N, the number of normal cells, also involve statistical parameters that are estimated by fitting to the data on nasal SCCs from two large rat inhalation bioassays of formaldehyde (Kerns *et al.*, 1983; Monticello *et al.*, 1996). The parameters α_N, α_I, β_I, μ_N and μ_I are characterized as a function of the local regional flux of formaldehyde into the rat nasal tissue using a CFD model (Kimbell *et al.*, 2001a). Estimates of the division rate of normal cells (α_N) are obtained by applying a formula of Moolgavkar and Luebeck (1992) to the Monticello *et al.* (1991, 1996) cell labeling data in formaldehyde-exposed rats. Division rates for exposure concentrations not applied in the rat experiments are calculated by linear interpolation or extrapolation.

In the rat modeling, Conolly *et al.* (2003) consider two models for α_N, the division rate of normal cells (Fig. 1), based on data from cell labeling experiments

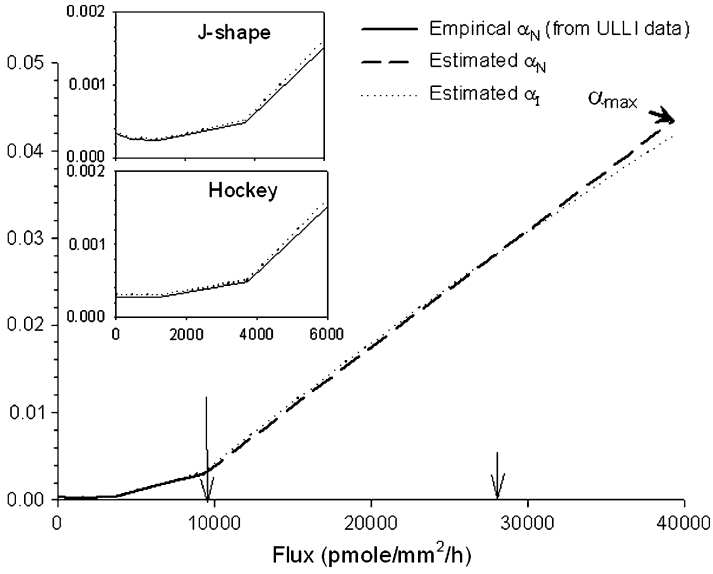

Fig. 1. Dose response of rat cell division rates in Conolly *et al.* (2003). Empirically derived values of α_N (time-weighted average over six sites) from Table 1 in Conolly *et al.* (2003) and optimized parameter values from their Table 4 were used. Main panel is for the J-shape dose response. Insets show J shape and hockey stick representations at low end of flux range. Long arrow denotes upper end of flux range for which the empirical unit-length labeling data are available for use in the two-stage model. α_{max} is the value of α_N at the maximum formaldehyde flux delivered at 15 p.p.m. exposure and was estimated by optimizing against the tumor incidence data. $\alpha_I < \alpha_N$ for flux greater than value indicated by small vertical arrow. $\beta_I = \alpha_N$ at all flux values.

Monticello *et al.* (1991, 1996). In the J-shape model, the division rate decreases below the basal value at low values of flux. The hockey stick model contains a threshold value of flux, below which formaldehyde has no effect upon cell division rates. The J shape represents the time-weighted average over nasal sites of division rate constants calculated from the cell labeling data. The fit of the hockey stick model to the cell replication data was not statistically significantly different from that of the J shape (Gaylor *et al.*, 2004).

No data are available on the division or death rates of initiated cells (i.e. on α_I or β_I). The flux-specific rate of division of initiated cells, α_I, is estimated from the rate for normal cells by the empirical formula,

$$\alpha_I = \alpha_N\{\text{multb} - \text{multfc} \times \max[\alpha_N - \alpha_{N\text{basal}}, 0]\}, \quad (1)$$

where the statistical parameters, multb and multfc, are estimated by fitting to the rat bioassay data. The rate of cell death of initiated cells is assumed to be equal to the rate of division of normal cells, i.e. $\beta_I = \alpha_N$ for all values of formaldehyde flux. The dose responses for normal and initiated cell replication rates considered in Conolly *et al.* (2003) are shown in Fig. 1.

The mutation rates of normal cells, μ_N, and of initiated cells, μ_I, are assumed to be equal and linearly related to DPX, i.e.

$$\mu_N = \mu_I = \mu_{\text{basal}} + \text{KMU} \times \text{DPX}. \quad (2)$$

In the rat model (Conolly *et al.*, 2003), both the basal mutation rate, μ_{basal}, and the linear slope, KMU, are estimated from fitting to the rat data. The concentrations of DPX are estimated from applying a pharmacokinetic (PBPK) model to experimental rat data (Conolly *et al.*, 2000).

The delay, *D*, from the occurrence of the first malignant cell until the resulting death of the animal is also an estimated parameter. In summary, the rat model involves six statistical parameters estimated from fitting to the rat bioassay data: $\alpha_{\max}$, multb, multfc, μ_{basal}, DPX and *D*.

Our implementation of the Conolly *et al.* (2003) rat model (Subramaniam *et al.*, 2007) corrected some errors in the Conolly *et al.* implementation and made some other fairly minor changes that we believed led to an improvement in the model. However, even with these corrections and changes, we were still able to reproduce the results of the Conolly *et al.* implementation in all major respects.

The Conolly *et al.* (2004) human model is conceptually very similar to the rat model, although rather than considering only nasal tumors, it is used to predict the risk of all human respiratory tumors. Local formaldehyde flux to respiratory tissue is estimated by a CFD model for humans (Kimbell *et al.*, 2001a; Overton *et al.*, 2001). The rat cell-labeling data are used in the same manner as in the rat model to estimate cell division rates in the human, except that a human estimate is used for the fraction of cells capable of dividing in the Moolgavkar and Luebeck (1992) formula. DPX in humans is estimated by scaling up from experiments in rats and monkeys.

The delay, *D*, is fixed at 3.5 years, based on a fit to the incidence of lung cancer in a cohort of British doctors (Doll and Peto, 1978). The two other parameters that affect the background rate of cancer (multb and μ_{basal}) are estimated by fitting to US cancer incidence or mortality data. Since $\alpha_{\max}$, multfc and KMU do not affect the background cancer rate, they cannot be estimated from the human data. Therefore, in Conolly *et al.* (2003, 2004), $\alpha_{\max}$ and multfc are assumed to have the same values in humans as in rats, and the human value for KMU is obtained by assuming that the ratio KPX $\equiv$ KMU/μ_{basal} is invariant across species. Thus,

$$\text{KMU}_{(\text{human})} = \text{KMU}_{(\text{rat})} \times \frac{\mu_{N\text{basal(human)}}}{\mu_{N\text{basal(rat)}}}. \quad (3)$$

RESULTS

Our earlier evaluation of the rat model (Subramaniam *et al.*, 2007) found that the ratio KMU of the DPX coefficient to the basal mutation rate was very sensitive to the choice of control data included in the model. Since this ratio is assumed to be the same in rats and humans (equation (3)), we hypothesized that the choice of control data could also have a large impact upon estimates of human risk. In this section, we evaluate quantitatively the impact of different control groups upon estimates of additional human risk.

No data are available on the rates of division and death of initiated cells. Conolly *et al.* (2003, 2004) assumed a mathematical expression (equation (1)) for the division rates of initiated cells that is based upon their estimate of the division rates of normal cells and showed that this expression, after estimating the two parameters (multb and multfc in equation (1)) by optimizing the fit to the rat tumor data, provided a reasonable fit to these data. However, they did not conduct a sensitivity analysis to determine whether other expressions would also fit the rat data but lead to different estimates of human risk. In this section, we modify their assumptions and consider the effect both upon the fit to the rat data and the predictions of additional human risk.

Fit of model to human data

Conolly *et al.* (2004) developed estimates of additional human risk of respiratory cancer from formaldehyde exposure in US males, using Surveillance

Epidemiology and End Results (SEER) data, and separately in smokers and non-smokers. In the limited sensitivity analysis that is the focus of the present work, to illustrate the points we wish to make, we only consider risks to the general US population from constant lifetime exposure to various levels of formaldehyde under the Conolly *et al.* (2004) environmental scenario (8 h day^{-1} sleeping, 8 h day^{-1} sitting and 8 h day^{-1} engaged in light activity). Figure 2 shows our fit of the formaldehyde human model to the SEER data for incidence of respiratory cancer in males and females combined for 2000–2002 (SEER, 2005; see supplementary Table A1, available at *Annals of Occupational Hygiene* online). The model does not predict the downturn in human respiratory cancer past age 80, and, as we believe Conolly *et al.* (2004) also did, we only fit the model to the incidence data between the ages of 20 and 80. Fits based on the hockey stick and J-shape models were identical, and of the three estimated parameters (μ_{basal}, multb and D), only the estimate of μ_{basal} differed between the two models (Table 1).

Whereas Conolly *et al.* (2004) used a fixed value for the delay, D, in our implementation of the human model, we estimated D by fitting the model to the SEER data (see supplementary material, available at *Annals of Occupational Hygiene* online) in the same way that D was estimated in the rat model by fitting to the rat tumor data. Our estimate of D = 21.8 years is considerably larger than the value of 3.5 years assumed by Conolly *et al.* (2004), which was based on a finding that, among a cohort of British doctors who began smoking at an average age of 19 years, the incidence of lung cancer was proportional to (age − 22.5) years raised to a power (Doll and Peto, 1978). In reviewing this approach, it seemed to us that there was no strong basis for assuming that the difference of 22.5 − 19 = 3.5 years represents the delay from the occurrence of the initial malignant cell to death. The Doll and Peto (1978) model is a statistical description of the lung cancer data, and the authors do not suggest such a biological interpretation for a 3.5 years delay. Our estimate of D in humans is much nearer to the same fraction of a human life span (22 years/75 years = 0.3) as the estimate of D in rats is to the fraction of a rat life span (300 days/730 days = 0.4) than is the 3.5 years delay assumed by Conolly *et al.* (2004) (3.5 years/75 years = 0.05). Estimating D made a small, but not dramatic, improvement in the fit to the SEER data. Likewise, estimating D made no meaningful difference upon estimates of human risk, and, as to be discussed later, our implementation was able to reproduce the Conolly *et al.* risk estimates very closely.

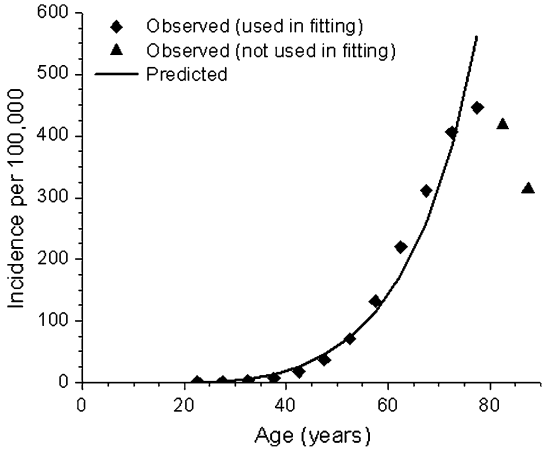

Fig. 2. Fit obtained in this analysis of Conolly *et al.* (2004) human model to SEER incidence data (Table A1, available at *Annals of Occupational Hygiene* online) (SEER, 2005) on human respiratory cancer.

Effect of alternative background rates of nasal tumors in rats

In their implementation of the rat model, Conolly *et al.* (2003) augmented the rat formaldehyde bioassay data by incorporating data on background rates of nasal SSC in 7684 historical control rats from NTP bioassays. As discussed in our evaluation of the rat model (Subramaniam *et al.*, 2007), these controls came from different rat colonies and from experiments conducted in different laboratories over a wide span of years, so it is clearly problematic to assume that background rates in these historical control animals are the same as those in the concurrent control group. There are considerable differences among the background tumor rates of SCC in all NTP controls (13/7684 = 0.0017), NTP inhalation controls (1/4551 = 0.0002) and concurrent controls (0/341 = 0.0) (Subramaniam *et al.*, 2007). The rate in all NTP controls is significantly higher than that in NTP inhalation controls (P = 0.01, Fisher's exact test). Subramaniam *et al.* (2007) showed these differences to significantly impact the calibration of the rat model. The biggest influence is on the estimated basal mutation rate in rats, $\mu_{Nbasal(rat)}$, which, in turn, influences the estimated mutation effect in humans through equation (3).

To understand the effect of the decision by Conolly *et al.* (2004) to include data on NTP historical animals in their model, we conducted analyses that included (i) the same controls as used by Conolly *et al.* (2004) (i.e. concurrent controls plus all NTP controls), (ii) concurrent controls plus controls from NTP inhalation studies and (iii) only concurrent controls. Table 1 contains parameter estimates for both the animal and human models obtained from these analyses [The estimates in this table differ from those in Table 3 of Subramaniam *et al.* (2007) because the latter table was obtained using a different model for DPX formation than was used by Conolly *et al.* (2003, 2004).]. Each set of control data was applied with both the J-shape and hockey stick models for

Table 1. Parameter estimates from fits to rat and human data used to estimate human risk[a]

Control animals	J shape for cell replication				Hockey stick for cell replication			
	All NTP historical		NTP inhalation historical	Concurrent only	All NTP historical		Inhalation historical	Concurrent only
	This analysis	Conolly *et al.*	This analysis	This analysis	This analysis	Conolly *et al.*	This analysis	This analysis
Results from fitting rat model								
Log-likelihood	−1692.64	−2131.5	−1493.46	−1474.39	−1693.68	−2133.1	−1493.30	−1474.39
$\mu_{N\,basal}$	1.87×10^{-6}	1.35×10^{-6}	5.12×10^{-7}	5.31×10^{-9}	2.12×10^{-6}	1.47×10^{-6}	9.13×10^{-7}	0
KMU	4.74×10^{-7}	0	3.67×10^{-6}	5.05×10^{-6}	0	0	2.57×10^{-6}	5.05×10^{-6}
KPX (KMU/$\mu_{N\,basal}$)[b]	0.254	0	7.18	$9.52 \times 10^{+2}$	0	0	2.81	INF[c]
KPX 90% CI[b]	(0, 0.973)	(0, 0.606)	(1.23, 18.4)	(1.53, INF[c])	(0, 0.614)		(0.411, 17.4)	(1.69, INF[c])
D_0 (days)	239	291[d]	242	244	238	297[d]	237	244
D_{OF} (days)	66.8		70.7	69.3	67.3		68.8	69.5
multb	1.05	1.07	1.06	1.08	1.05	1.07	1.06	1.08
multfc[b]	1.86	2.58	2.52	3.35	1.77	2.52	2.55	3.35
α_{max} (h^{-1})[b,e]	0.045	0.0435	0.045	0.045	0.045	0.0435	0.045	0.045
Results from fitting human model								
$\mu_{N\,basal}$	6.21×10^{-9}	5.71×10^{-9}	6.21×10^{-9}	6.21×10^{-9}	7.53×10^{-9}	7.24×10^{-9}	7.53×10^{-9}	7.53×10^{-9}
KMU[f]	1.58×10^{-9}	0	4.46×10^{-8}	5.91×10^{-6}	0	0	2.11×10^{-8}	INF
D (years)	21.8	3.5	21.8	21.8	21.8	3.5	21.8	21.8
multib	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02

CI, confidence interval.
[a]Estimates are provided based on including all NTP control animals in the analysis, including NTP inhalation controls or only including concurrent controls, and compared with estimates obtained by Conolly *et al.* (2004).
[b]Parameter cannot be estimated from the human data and the rat value is used in the human model.
[c]'INF' indicates that the value cannot be bounded (is theoretically infinite).
[d]D_o and D_{of} were not separately estimated by Conolly *et al.* (2004) but only their sum.
[e]An UB for α_{max} of 0.045 was used, as that value was assumed to be the largest plausible rate of cell division.
[f]Although 'KMU' cannot be independently estimated from the human data, it assumes a different value in the human model because it depends upon the human value for $\mu_{N\,basal}$ (see equation (3)).

cell replication for a total of six analyses. The top portion of Table 1 contains the parameter estimates obtained from fitting the rat model, and the bottom portion contains the parameters that affect background tumor rates and which consequently were obtained from fitting the human model to the background human data. Parameters α_{max}, multfc and KMU were estimated in exactly the same manner as in Conolly *et al.* (2004) (see supplementary material, available at *Annals of Occupational Hygiene* online). Table 1 also contains 90% statistical confidence intervals for KPX. For comparison, parameter values from Conolly *et al.* (2003, 2004) (which were obtained using all NTP controls) are also listed. Except for minor differences, our results in Table 1 using all NTP controls are similar to those obtained by Conolly *et al.* (2003, 2004). The reasons for these minor differences have been addressed in our rat paper Subramaniam *et al.* (2007).

To determine how estimates of human risk obtained from our implementation of the human model compared to those in Conolly *et al.* (2004), we compared estimates of human risk from constant lifetime formaldehyde exposure derived using our implementation with all NTP controls (columns 1 and 5 of Table 1) to corresponding estimates in Conolly *et al.* (2004). This paper only presents estimates of additional human risk derived using UB estimates of KMU. In order to compare with these estimates, we replaced the MLE of KPX = KMU/μ_{basal} by its 95% UB in computing our estimates. Throughout the range of formaldehyde exposures considered by Conolly *et al.* (from 0.001 to 1 p.p.m.), our estimates of additional risk differ from their estimates only by factors of 1.3 (J-shape model) and 1.7 (hockey stick model). These factors are quite small compared to the overall uncertainty in low-dose risk estimates in general and demonstrate that the differences in our implementation of the Conolly *et al.* (2004) model, as described earlier (see also supplementary material, available at *Annals of Occupational Hygiene* online), did not have important effects upon estimates of low-dose risk.

Unlike the analyses that include all NTP controls, Table 1 shows that MLE estimates of the ratio KPX = KMU/μ_{basal} are statistically significantly greater than zero (and consequently so are estimates of the mutational coefficient, KMU) when either inhalation NTP controls or only concurrent controls are used. The 95% UBs for KPX = KMU/μ_{basal} obtained using inhalation NTP controls are 20–30 times higher than the comparable bounds obtained using all NTP controls. Moreover, when only concurrent controls are used, this parameter cannot be bounded. (The UB is undefined, or infinite, due to the fact that the estimate of μ_{basal} cannot be bounded away from zero.) Since the estimate of KMU in the human (the parameter that determines the mutational effect) is computed by multiplying the animal ratio, KPX = KMU/μ_{basal} by the human μ_{basal}, a large value for KPX produces a large mutational effect in the human model.

We now consider differences in additional human risk resulting from the use of different control groups for modeling the rat bioassay data. Figure 3 contains predictions resulting from the analyses shown in Table 1 of additional risk in humans from constant lifetime exposure to various levels of formaldehyde. The lowest dotted curve in this figure represents the highest estimates of human risk developed by Conolly *et al.* (2004), which resulted from use of the hockey stick model for cell division rates in

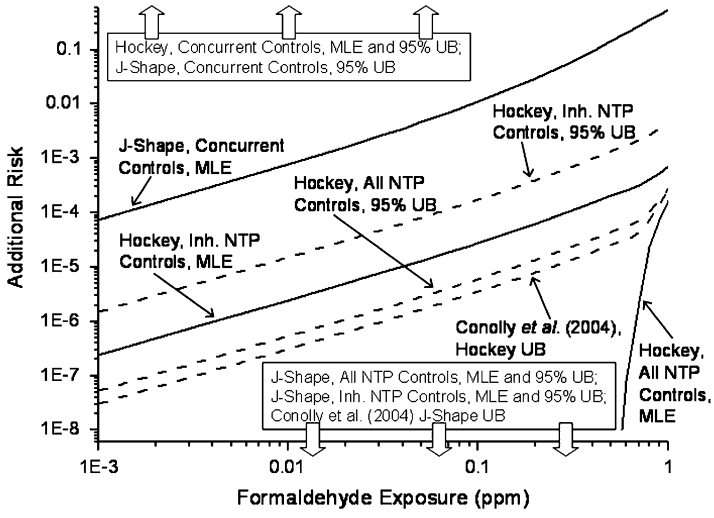

Fig. 3. Estimates of additional human risk of respiratory cancer by age 80 from lifetime exposure to formaldehyde obtained using different control groups of rats.

conjunction with the statistical UB for KMU. As indicated in Fig. 3, corresponding estimates based on the J-shape model were all negative for exposures <1 p.p.m. Figure 3 also shows the MLE risks predicted by the six analyses shown in Table 1, along with (analogous to the risk estimates obtained by Conolly *et al.*, 2004) the estimates of human risk obtained in each case by replacing the MLE of KPX = KMU/μ_{basal} by the corresponding 95% UB. Note that the close correspondence of the two dotted curves confirms, at least for the hockey stick model for cell replication, our previous observation that our implementation essentially reproduced the risk estimates obtained by Conolly *et al.* (2004).

Figure 3 also shows that the choice of controls to include in the rat model can make an enormous difference in estimates of additional human risk. For the J-shape model, both MLE estimates and those based upon the 95% UB on KPX = KMU/μ_{basal} are negative for formaldehyde exposures <1 p.p.m., when either all NTP controls or only NTP inhalation controls are included. However, when only concurrent controls are used in the model, the MLE from the J-shape model is positive and is more than three orders of magnitude higher than the highest estimates obtained by Conolly *et al.* (2004), and estimates based upon the 95% UB on KPX are unboundedly large, as indicated by the block arrows at the top of Fig. 3.

Choice of control animals makes a similarly large difference in the estimates of additional risk obtained using the hockey stick model. Using all NTP controls, the estimates based on the MLE are zero for exposures less than ~0.5 p.p.m., but, as noted above, the estimates based on the 95% UB on KPX closely approximate the Conolly *et al.* (2004) UB estimates. However, if only inhalation controls are added, the MLE are about seven times larger than the Conolly *et al.* (2004) UB estimates, and the estimates based on the 95% UB on KPX are ~50 times larger than the Conolly *et al.* (2004) estimates. If only concurrent controls are used, both the MLE estimates and those based on the 95% UB on KPX are unboundedly large.

Effect of alternative assumptions regarding the rate of replication of initiated cells

Conolly *et al.* (2004) estimated the replication rate of initiated cells, α_I, based on the rate in normal cells, α_N, using equation (1). The dose response of initiated cells with formaldehyde flux predicted by this formula is very similar to either the J shape or hockey stick shape assumed for normal cells (Fig. 1). In both cases, for high values of flux, $\alpha_I < \alpha_N$. Consequently, since $\beta_I = \alpha_N$ at these flux levels, we also have $\alpha_I < \beta_I$.

Since no data or information are available on division rates of initiated cells to inform a likely relationship between normal and initiated cell division rates for cells in the respiratory epithelium, parameters in equation (1) were estimated based solely on fitting the tumor probability predicted by the rat model to the rat tumor data. It is therefore important to explore alternative assumptions about these rates. In addition to determining how estimates of additional risk are affected, each alternative should be evaluated with respect to biological plausibility and concordance of the modified tumor model with the underlying rat and human tumor data.

We considered two alternatives for each of the J-shape and hockey stick models. Figure 4 shows the hockey stick model for initiated cells in rats. In the first modification to the hockey stick model (Hockey Mod 1), rather than having a threshold at a flux of 1240 μm m^{-2} h^{-1}, the division rate increases linearly with increasing flux until the graph intersects the original curve at 4500 μm m^{-2} h^{-1} where it then assumes the same value as in the original curve for larger values of flux. The second modification (Hockey Mod 2) is similar, except the modified curve intersects the original curve at a flux of 3000 μm m^{-2} h^{-1}.

Figure 5 shows the rat J-shape model for initiated cells. In the first modification to this dose response (J-shape Mod 1), rather than having a J shape, the division rate of initiated cells remains constant at the basal value until the original curve rises above the basal value and has the same value as the original curve for larger values of flux. In the second modification (J-shape Mod 2), the J shape is retained, but somewhat mitigated. In this modification, the division rate initially decreases in a linear manner similar to that of the original model, but with a less negative slope, until it intersects the original curve at a flux of 1240 μm m^{-2} h^{-1} where it then follows the original curve for higher values of flux. These modifications to the cell replication rates assumed by Conolly *et al.* (2004) for initiated cells are small compared to the variation in the measured values of cell replication rates in normal cells (Fig. 6).

Each of the modified models in Figs 4 and 5 was applied in the version of the tumor models that employed all NTP controls. We continued to use all NTP historical controls since the purpose of this work is only a sensitivity analysis (as opposed to developing alternate credible risk estimates). Since none of these modifications affect the basal rate of cell division, they likewise have no effect upon the fit to the human background data. Table 2 shows the changes in the log-likelihoods for the rat tumor data resulting from these modifications. The largest change occurs with the Hockey Mod 2, and the reduction in the log-likelihood in this case is only 0.64, which indicates a very modest effect of the modification upon the fit of the model to the rat tumor data. This conclusion is borne out graphically in Fig. 7. This figure shows curves of the cumulative probability of a rat dying from a nasal SCC by a given age for bioassay exposure groups of 6, 10 and

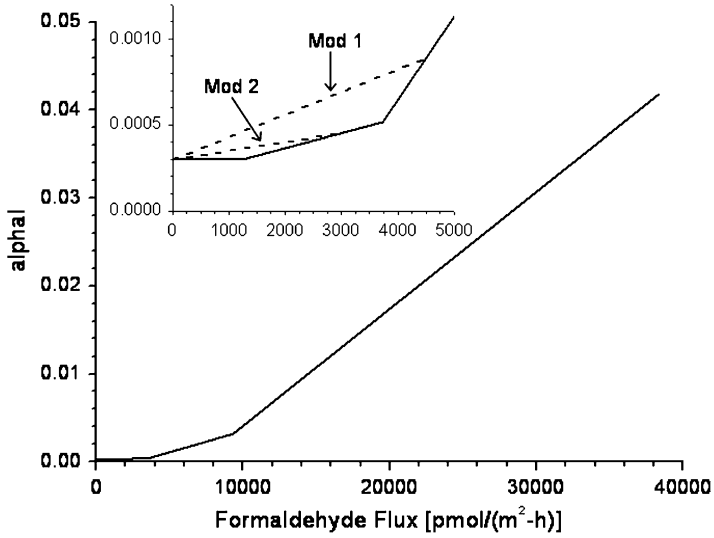

Fig. 4. Conolly *et al.* (2003) hockey stick model for division rates of initiated cells in rats, and 2 modified models.

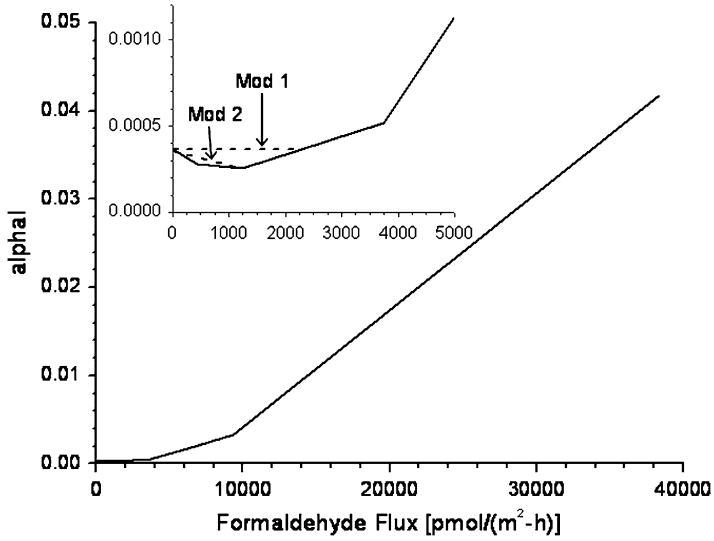

Fig. 5. Conolly *et al.* (2003) J-shape model for division rates of initiated cells in rats, and 2 modified models.

15 p.p.m. These estimates are based upon the form of the formaldehyde model that uses all NTP controls and the hockey stick model. For comparison purposes, the corresponding Kaplan–Meier (nonparametric) estimates of the probability of death from a nasal tumor are also shown. What is not readily apparent from the graph is that two sets of probabilities are graphed: the original unmodified ones and the ones obtained using Hockey Mod 1. The changes in the tumor probability resulting from this modification are so slight that the two models cannot be readily distinguished in this graph. In fact, the largest change in the tumor probability resulting from this modification, for any dose group and any age up through 900 days is <0.002, a change so small that it would be impossible to detect, even in the largest bioassays ever conducted. The changes in tumor probability resulting from the remaining three modifications are even smaller (Table 2). These comparisons were made without reoptimizing the likelihood, which would have made the fit of modified models even better. Thus, these modifications to the models for the division rates of initiated cells caused an inconsequential change in the fit of the model-predicted tumor incidence to the animal tumor data.

Figure 8 contains graphs of the additional human risks estimated by applying these modified models for α_I using all NTP controls, compared to those obtained using the original Conolly *et al.* (2004) model.

It should be noted that since KPX = KMU/μ_{basal} is estimated as zero using either the J shape or hockey stick model (Table 1), no mutational component is included in these graphs. The additional risks predicted by the hockey stick model in Conolly *et al.* (2004) drop off rapidly with decreasing parts per million of formaldehyde down to a threshold of ~0.5 p.p.m. and remain zero below this threshold. The additional risks predicted by the original J-shape model (not pictured) also drop off rapidly with decreasing parts per million and assume negative values below ~1.5 p.p.m. However, each of the four modified models presents a very different picture. At low exposures, these risks are three to four orders of magnitude larger than the largest estimates obtained by Conolly *et al.* (2004). At 0.1 p.p.m., the exposure at which Conolly *et al.* (2004) claimed the risk was *de minimis*, the additional risks predicted by these models modified to evaluate model sensitivity are all >0.01. Since the additional risk from the two-stage model is a continuous function of the division rate of initiated cells, it is clear that by making suitable small perturbations to the these rates, one can obtain any additional risk ranging from negative values up to at least four orders of magnitude higher than the conservative estimates of Conolly *et al.* (2004), while fitting the underlying data equally as well as the original model.

Table 2. Changes in log-likelihood using all NTP controls resulting from effect of modifications[a] to division rates of initiated cells upon fit to data

Modification	Original log-likelihood	Modified log-likelihood	Difference
Hockey stick Mod 1	−1693.68	−1694.32	0.64
Hockey stick Mod 2	−1693.68	−1693.75	0.07
J-shape Mod 1	−1692.64	−1692.78	0.14
J-shape Mod 2	−1692.64	−1692.69	0.05

[a]See text and Figs 4 and 5 for descriptions of the modifications.

DISCUSSION

This paper presents a limited sensitivity analysis of only two aspects of the formaldehyde model: (i) assumptions regarding the dose response for the division rate of initiated cells and (ii) assumptions regarding the incorporation of historical control data into the rodent model. This analysis shows that the estimates of additional human risk obtained from the formaldehyde model are highly sensitive to these assumptions. Other reasonable assumptions are also consistent with the available data, but lead to estimates of human risk that are far higher than those in Conolly *et al.* (2004). Consequently, the estimates of human risk obtained by Conolly *et al.* (2004) cannot be considered conservative.

Model estimates of low-dose risks invariably require assumptions about the shape of dose–response curves at low doses. Whereas purely statistical models make assumptions about the shape of the frank tumor response, models like the formaldehyde model incorporate assumptions regarding the dose response for upstream steps in the carcinogenic process. We performed sensitivity analyses to examine the range of risks predicted by models with assumptions different from those of Conolly *et al.* about these upstream steps, but which are also consistent with the data.

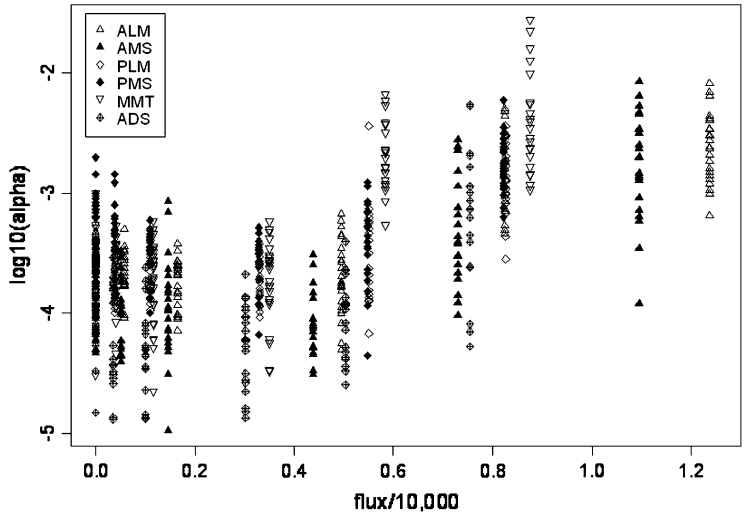

Fig. 6. Logarithm of normal cell replication rate versus formaldehyde flux for the F344 rat nasal epithelium. Each point represents a measurement for one rat, at one nasal site at one of four sacrifice times (13, 26, 52, 78 weeks). Legend denotes the nasal sites as in Monticello *et al.* (1996).

To this end, we made minor modifications to the assumed division rates of initiated cells in Conolly *et al.* (2004) while keeping all other aspects of the model and input data unchanged (Figs 4 and 5) and considered the resulting change to estimates of additional human risk (Fig. 8). This work demonstrates that perturbing the dose response for α_I slightly without changing β_I can result in a very large increase (by three to four orders of magnitude) in estimated additional human risk, while making essentially no difference in the fit to the available data (Table 2 and Fig. 7). In the case of the J-shape model for α_I, our sensitivity analyses indicate that it is not necessary to remove the J shape to produce these large increases in estimated additional human risk; keeping the J shape but simply mitigating it slightly is sufficient to produce positive estimates of additional risk that are >1000-fold higher than the Conolly *et al.* (2004) estimates (Fig. 5, J-shape Mod 2).

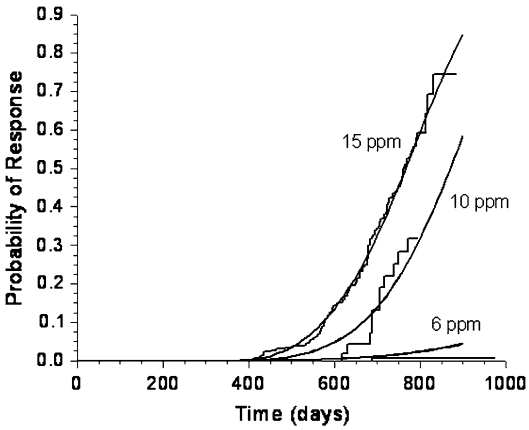

Fig. 7. Model estimates of probability of fatal tumor in rats compared to Kaplan–Meier estimates. Model estimates are derived from hockey stick model fit to concurrent data (Table 1) and a modification to the hockey stick data (Fig. 4, Mod 1). The two sets of model estimates are so similar that they cannot be distinguished on this graph. Only estimates for formaldehyde exposures to 6, 10 and 15 p.p.m. are presented, as there were no tumors in rats at lower exposures.

A crucial result in the Conolly *et al.* (2004) modeling was that the optimal value for KMU in equation (2) was zero, which implies no mutational effect of formaldehyde. As a consequence, the optimal value of additional risk at low exposures was negative for the J-shape model and zero for the hockey stick model. To obtain a conservative estimate of risk in the hockey stick model, Conolly *et al.* (2004) increased the value for KMU by an amount such that the fit to the tumor data was not changed significantly, thereby introducing a small linear formaldehyde-induced contribution to the mutational probability. The resulting additional human risk, although then positive, was concluded to be *de minimis* at environmental levels of human exposure (Conolly *et al.*, 2004). However, this conclusion does not consider the potential effect of changes in other parameters or plausible variations in model specification, as were partially explored in the present sensitivity analyses. It is important to note that the large increases in risk described in the previous paragraph are from models in which it was assumed that formaldehyde has no mutational effect. Thus, even without invoking formaldehyde's mutagenic action, a much higher low-dose risk than obtained by Conolly *et al.* (2004), and which is also consistent with the tumor data, cannot be ruled out.

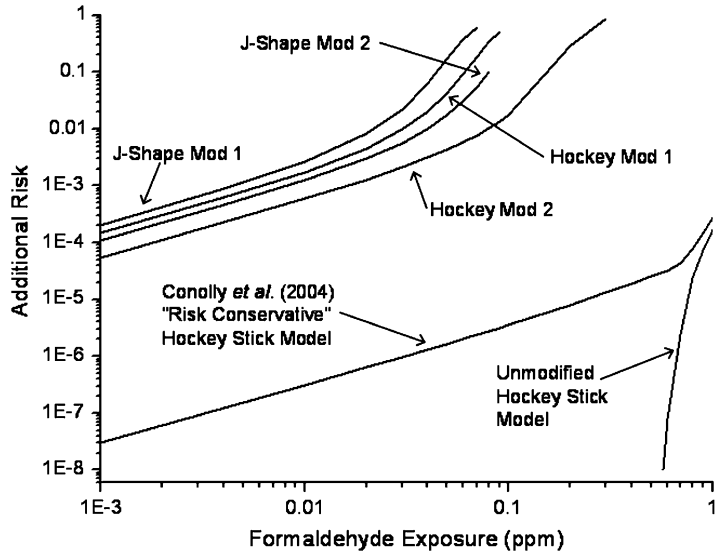

Fig. 8. Estimates of additional human risk of respiratory cancer by age 80 from lifetime exposure to formaldehyde obtained using modified cell division rates for initiated cells (Figs 4 and 5).

In general, the rate of growth of clones of intermediate cells depends primarily upon $\alpha_I - \beta_I$, the difference in the birth rate and death rate of intermediate cells (Schulte-Hermann *et al.*, 1999). This difference is an important determinant of risk as predicted by the two-stage model. There is some evidence that α_I and β_I rise or fall concomitantly (Grasl-Kraupp *et al.*, 1997; Schulte-Hermann *et al.*, 1999; Grasl-Kraupp *et al.*, 2000). Apart from this broad determination, even assuming that it is relevant for formaldehyde-exposed respiratory epithelial cells, there is no indication as to how tightly one might expect these rates to track each other. Furthermore, it appears that $\alpha_I - \beta_I$ will likely have a strong temporal dependence, as suggested by the work of Grasl-Kraupp *et al.* (2000). The site and temporal variability in the measured labeling index in the formaldehyde rodent bioassays, and thus in the derived normal cell replication rates, was recognized to be large by Conolly *et al.* (2003). However, this variability was not characterized for input to their two-stage modeling and is therefore not expressed in (nor does it influence) the model-derived values of α_I. Thus, in summary, we have seen nothing in the literature, either generally or specifically for formaldehyde, to rule out the small increases in $\alpha_I - \beta_I$ resulting from our modifications.

The increase in $\alpha_I - \beta_I$ stemming from our modifications at low flux values are much smaller than the increase assumed in the Conolly *et al.* (2004) model at higher flux values. In the Conolly *et al.* (2004) hockey stick model, $\alpha_I - \beta_I$ reaches a maximum of 5.0×10^{-4} h^{-1} at a flux of 17190 pmole mm^{-2}-h in the human respiratory tract, and then decreases with increasing flux eventually becoming negative. By comparison, the largest difference in $\alpha_I - \beta_I$ in the flux region where we modified the model is 2.9×10^{-4} h^{-1} for Hockey Mod 1 and 7.7×10^{-5} h^{-1} for Hockey Mod 2. Likewise, in the Conolly *et al.* (2004) J-shape model, $\alpha_I - \beta_I$ reaches a maximum of 5.2×10^{-4} h^{-1}, whereas, in the range where we modified α_I, the maximum of $\alpha_I - \beta_I$ is only 1.2×10^{-4} h^{-1} for J-shape Mod 1 and 6.1×10^{-5} h^{-1} for J-shape Mod 2. Thus, while we do not see any compelling argument for assuming the values of $\alpha_I - \beta_I$ resulting from our modifications that are implausibly large, if there were such an argument, it should apply with even greater force to the assumptions about $\alpha_I - \beta_I$ in the original Conolly *et al.* (2004) model.

There are other biological considerations that motivate our exercise. The averaged cell replication rate constants as tabulated in Table 1 of Conolly *et al.* (2003) (for various exposure concentrations and corresponding average formaldehyde flux values in the F344 rat nose) demonstrate an increase over baseline values only at exposure concentrations of 6 p.p.m. and higher. Increased cell proliferation at these concentrations of formaldehyde, whether transient or sustained, have been associated in the literature with epithelial response to the cytotoxic properties of formaldehyde (Monticello *et al.*, 1991, 1996; Monticello and Morgan, 1997; Conolly *et al.*, 2002), and the labeling data are considered to show a lack of cytotoxicity and regenerative cell proliferation in the F344 rat at exposures of 2 p.p.m. and below (Conolly *et al.*, 2002). In the Conolly *et al.* modeling, it is further assumed that the formaldehyde flux levels at which cell replication exceeds baseline rates remains essentially unchanged for initiated cells not only for the rodent but also when extrapolated to the human. These assumptions need to be viewed in the context of the uncertainty and variability in the data discussed further in Subramaniam *et al.* (2008). In addition, it may be noted that there are some limited data indicating that exposure to formaldehyde can result in increased cell replication at doses far below those that are considered to be cytotoxic. Tyihak *et al.* (2001) treated different human cell lines in culture to various doses (0.1–10 mM) of formaldehyde and found that the mitotic index increased at the lowest dose of 0.1 mM. These findings considered along with human population variability and susceptibility (for example, polymorphisms in alcohol dehydrogenase-3, Hedberg *et al.*, 2001) indicate that it is necessary to consider the possibility of small increases in the human α_I over baseline levels at exposures well below those at which cytotoxicity-driven proliferative response is thought to occur. This intent is represented by the increments in Hockey Mod1 and Mod2. Although we replaced the threshold model for initiated cells in Conolly *et al.* with a model that had a small positive slope at the origin, we could also have shifted the resulting curves 'Mod1' and 'Mod2' slightly to the right along the flux axis, so as to introduce a threshold for α_I and without materially affecting the risk estimates resulting from these modified curves. Thus, the assumption of a linear no-threshold response is not an essential feature of our modifications to the hockey stick model, as clearly threshold models exist that would produce essentially the same effect.

Heck and Casanova (1999) have provided arguments to explain that the formation of DPX by formaldehyde leads to inhibition of cell replication. As per this effect, the formation of DPX is thought to activate a checkpoint in the cell cycle so as to enable DNA repair; i.e. if this effect alone is considered, normal cell replication rate of the exposed cells would be less than the baseline rate. However, this hypothesis was posed for normal cells. If an initiated cell is created by a specific mutation that impairs this cell cycle control, the effect would be to mitigate the DPX-induced inhibition in cell replication, either partially or fully, depending on the extent to which the cell cycle control has been

disrupted. In the absence of data on initiated cells, the above argument provides biological motivation to the modification we applied to the J-shape model for cell division.

Although there are no data on division rates of initiated cells upon which to evaluate the modifications we made to these rates, we can gain some perspective by comparing them to the variability in the division rates obtained from the data on normal cells used to construct the formaldehyde model. As shown in Fig. 6 and discussed further in Subramaniam *et al.* (2008, Fig. 3), these data show roughly an order of magnitude variation in the cell replication rate at a given flux. As part of a statistical evaluation of these data, we calculated a standard deviation for the log-transforms of individual measurements of division rates of normal cells of 0.32. By comparison, the maximum change in the log-transform division rate of initiated cells resulting from hockey stick Mod 2 was only 0.20, and the average change would be considerably smaller. Thus, although there are no data for initiated cells, we can say that the modifications we introduce for initiated cells are extremely small in comparison to the dispersion in the data for normal cells.

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.

Another major assumption in the formaldehyde modeling (and which was retained in our modifications) is that initiated cell death rates (β_I) are equal to the replication rates of normal cells (α_N) for all values of flux. To evaluate the effect of this assumption, we also made small changes to the death rates of initiated cells and obtained similarly large values for estimates of additional human risk at low exposures (results not shown). Obtaining reliable data on cell death rates in the nasal epithelium appears to be an unusually difficult proposition (Monticello and Morgan, 1997; Hester *et al.*, 2003).

We want to make it abundantly clear that we are not claiming that the particular modifications we made to the Conolly *et al.* (2004) model resulted in more reliable or plausible models. Rather, we simply used these modifications to demonstrate that predictions of additional human risk from the Conolly *et al.* (2004) model are highly sensitive to small changes in model formulation that cannot be ruled out on either biological grounds or on the basis of concordance with the available data. Our analysis indicates that there is a continuum of alternative values of α_I that fit the available data and predict a corresponding continuum of low-dose additional human risks that range from negative values up through several orders of magnitude greater than any of the values estimated by Conolly *et al.* (2004). In other words, by making appropriate small changes in the assumptions by Conolly *et al.* regarding α_I (which are both consistent with biological knowledge and with the underlying data), one can get any additional risk one wants at low exposures, ranging from negative up to several orders of magnitude higher than the 'conservative estimates' obtained by Conolly *et al.* 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. Even if an inconsistency were found, that should only increase concern about the reliability of the CIIT model for assessing human risk. This extreme sensitivity of model results to small changes in α_I suggests that experimental data on initiated cells sufficiently accurate to restrict the human cancer risk projections from the two-stage formaldehyde model in a useful way are unlikely to be obtainable.

Our sensitivity analysis also evaluated the effect that use of different control groups for the formaldehyde animal bioassay data has on estimates of additional human risk. Since the incidence of nasal SCC in NTP inhalation controls is significantly different ($P = 0.01$) from that in the controls from all routes of exposure, we see no basis for preferring controls from studies that used all routes of exposure over controls from only inhalation studies. Replacing all NTP controls with NTP inhalation controls caused the additional human risk resulting from the UB estimate of the mutational effect to increase by ~50-fold (Fig. 3) over the UB obtained by Conolly *et al.* (2004).

Genetic and other time-related variation can lead to differences in tumor and survival rates across studies (Rao *et al.*, 1987; Haseman, 1992; Peddada *et al.* 2007). Studies also differ with regard to animal husbandry and pathology procedures. Given these differences, the inclusion of any type of historical controls is problematic and is thought to have limited value if these factors are not controlled for (Haseman, 1992).

However, if only concurrent controls are used, the formaldehyde human model does not place any UB on the risk. This is because the μ_{basal} value in rats cannot be bounded away from zero, which allows the mutational component in humans to be unboundedly large. Although it can be argued that the rate of SCC among the controls in the rat bioassay is probably not zero, it is also problematic to assume that this rate can be adequately represented by the background rate in NTP historical controls or even in NTP inhalation historical controls. If only inhalation controls from the NTP database are included, this leads to the rather bizarre situation in which, despite

all of the biological data that went into the formaldehyde model, the only piece of data that is keeping the human risk estimates bounded is a single tumor found among several thousand rats from NTP bioassays. This tumor came from the very first NTP inhalation study, dated in 1976, and the animals in this study were from Hazelton Laboratories, whereas the concurrent animals were all from Charles River Laboratories. Thus, it is problematic to assume that this tumor is representative of the risk of SCC in the formaldehyde bioassays.

The sensitivity analysis of the Conolly *et al.* (2003, 2004) formaldehyde model presented herein is limited, both in terms of the issues addressed and in terms of the range of alternatives investigated for those issues. A number of additional issues have been raised in this work that could be discussed, particularly concerning the validity of the data on the division rates of normal cells and how these data were incorporated into the model (Subramaniam *et al.*, 2008).

The human model for formaldehyde (Conolly *et al.*, 2004) has already been used by regulatory agencies to various degrees in supporting human health risk assessments or exposure standards for formaldehyde (Health Canada, 2001; Liteplo and Meek, 2003; BfR (Germany), 2006; the German MAK Commission, 2006; USEPA, 2006a,b). However, the analysis presented herein shows that the Conolly *et al.* model is highly sensitive to uncertain model assumptions, and other reasonable assumptions are equally compatible with the data but lead to much higher risks. These risks can be considerably higher than what would be obtained using standard statistical modeling approaches (USEPA, 2005). Thus, the formaldehyde human model cannot reasonably be used to narrow the range of low-dose human risk over that predicted by the standard regulatory approach.

The uncertainty in the Conolly *et al.* (2003, 2004) models is particularly acute because there are no data on initiated cells. However, it appears to us that any attempt to quantify low-dose human risk using animal data on intermediate steps in the carcinogenic process would be similarly uncertain. Nonetheless, the formaldehyde model is a very useful demonstration of a conceptual framework for integrating hybrid models for predicting regional toxicokinetics with toxicodynamic data. Such models can be valuable tools for hypotheses generation and testing and therefore can be important components in a risk assessment process that places importance on a chemical's mode of action. Our analysis shows that uncertainty and sensitivity analyses are essential tools in evaluating such models.

SUPPLEMENTARY MATERIAL

Supplementary materials and Table A1 can be found at http://annhyg.oxfordjournals.org/

FUNDING

US Environmental Protection Agency (IAG No. DW89939822-01-0) to K.S.C. and C.V.L. through its Office of Research and Development, under assistance agreement to the Department of Energy and via a contract with the Oak Ridge Associated Universities/Oak Ridge Institute for Science and Education by subcontract to ENVIRON Corporation.

Acknowledgements—We wish to express to the Hamner Institutes for Health Sciences (formerly CIIT) and to Dr Rory Conolly, our profound gratitude for providing the data needed for this work. We are indebted to Paul White, Danielle DeVoney, Chad Thompson and Paul Schlosser for useful discussions and Peter Preuss, John Vandenberg and David Bussard for their encouragement of this work. We thank Tammie Covington for her valuable assistance.

Disclaimer—The views expressed in this article are solely those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency or the views of those we acknowledge.

REFERENCES

BfR, Federal Institute of Risk Assessment, Germany. (2006) Toxicological assessment of formaldehyde. Opinion of BfR No. 023/2006. Available at http://www.bfr.bund.de/cm/290/toxicological_assessment_of_formaldehyde.pdf. Accessed 19 June 2008.

Casanova M, Morgan KT, Steinhagen WH *et al.* (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fundam Appl Toxicol; 17: 409–28.

Casanova M, Morgan KT, Gross EA *et al.* (1994) DNA-protein cross-links and cell replication at specific sites in the nose of F344 rats exposed subchronically to formaldehyde. Fundam Appl Toxicol; 23: 525–36.

Conolly RB, Lilly PD, Kimbell JS. (2000) Simulation modeling of the tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in F344 rats, rhesus monkeys, and humans. Environ Health Perspect; 108 (Suppl 5): 919–24.

Conolly RB, Kimbell JS, Janszen DB *et al.* (2002) Dose response for formaldehyde-induced cytotoxicity in the human respiratory tract. Regul Toxicol Pharmacol; 35: 32–43.

Conolly RB, Kimbell JS, Janszen D *et al.* (2003) Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol Sci; 75: 432–47.

Conolly RB, Kimbell JS, Janszen D *et al.* (2004) Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically motivated computational modeling of a combined rodent and human dataset. Toxicol Sci; 82: 279–96.

Crump KS. (1994a) Limitations of biological models of carcinogenesis for low-dose extrapolation. Risk Anal; 14: 883–6.

Crump KS. (1994b) Use of mechanistic models to estimate low dose cancer risks. Risk Anal; 14: 1033–8.

Doll R, Peto R. (1978) Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong nonsmokers. J Epidemiol Community Health; 32: 303–13.

Gaylor DW, Zheng Q. (1996) Risk assessment of nongenotoxic carcinogens based upon cell proliferation/death rates in rodents. Risk Anal; 16: 221–5.

Gaylor DW, Lutz WK, Conolly RB. (2004) Statistical analysis of non-monotonic dose-response relationships: research design and analysis of nasal cell proliferation in rats exposed to formaldehyde. Toxicol Sci; 77: 158–64.

Grafstrom RC, Curren RD, Yang LL *et al*. (1985) Genotoxicity of formaldehyde in cultured human bronchial fibroblasts. Science; 228: 89–91.

Grasl-Kraupp B, Ruttkay-Nedecky B, Müllauer L *et al*. (1997) Inherent increase of apoptosis in liver tumors: implications for carcinogenesis and tumor regression. Hepatology; 25: 906–11.

Grasl-Kraupp B, Luebeck G, Wagner A *et al*. (2000) Quantitative analysis of tumor initiation in rat liver: role of cell replication and cell death (apoptosis). Carcinogenesis; 21: 1411–21.

Haseman JK. (1992) Value of historical controls in the interpretation of rodent tumor data. Drug Inf J; 26: 191–200.

Health Canada. (2001) Priority substances list assessment report: formaldehyde. Available at http://www.ec.gc.ca/substances/ese/eng/psap/final/reports/formaldehyde.pdf. Accessed 19 June, 2008.

Heck H, Casanova M. (1999) Pharmacodynamics of formaldehyde: applications of a model for the arrest of DNA replication by DNA-protein cross-links. Toxicol Appl Pharmacol; 160: 86–100.

Heck H, Casanova M, Starr T. (1990) Formaldehyde toxicity—new understanding. Crit Rev Toxicol; 20: 397–420.

Hedberg JJ, Backlund M, Stromberg P *et al*. (2001) Functional polymorphism in the alcohol dehydrogenase 3 (ADH3) promoter. Pharmacogenetics; 11: 815–24.

Hester SD, Benavides GB, Yoon L *et al*. (2003) Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium. Toxicology; 187: 13–24.

Kerns W, Pavkov K, Donofrio D *et al*. (1983) Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res; 43: 4382–92.

Kimbell JS, Overton JH, Subramaniam RP *et al*. (2001a) Dosimetry modeling of inhaled formaldehyde: binning nasal flux predictions for quantitative risk assessment. Toxicol Sci; 64: 111–21.

Kimbell JS, Subramaniam RP, Gross EA *et al*. (2001b) Dosimetry modeling of inhaled formaldehyde: comparisons of local flux predictions in the rat, monkey, and human nasal passages. Toxicol Sci; 64: 100–10.

Liteplo RG, Meek ME. (2003) Inhaled formaldehyde: exposure estimation, hazard characterization, and exposure-response analysis. J Toxicol Environ Health B Crit Rev; 6: 85–114.

MAK Commission [German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area]. (2006) Formaldehyde, official English translation, occupational toxicants: critical data evaluation for MAK values and classification of carcinogens. Vol. 17. 163.

Monticello TM, Morgan KT. (1997) Chemically-induced nasal carcinogenesis and epithelial cell proliferation: a brief review. Mutat Res; 380: 33–41.

Monticello TM, Miller FJ, Morgan KT. (1991) Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. Toxicol Appl Pharmacol; 111: 409–21.

Monticello TM, Swenberg JA, Gross EA *et al*. (1996) Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res; 56: 1012–22.

Moolgavkar S, Knudson AJ. (1981) Mutation and cancer: a model for human carcinogenesis. J Natl Cancer Inst; 66: 1037–52.

Moolgavkar SH, Luebeck EG. (1992) Interpretation of labeling indices in the presence of cell death. Carcinogenesis; 13: 1007–10.

Moolgavkar S, Venzon D. (1979) Two-event model for carcinogenesis: incidence curves for childhood and adult tumors. Math Biosci; 47: 55–77.

Moolgavkar SH, Dewanji A, Venzon DJ. (1988) A stochastic 2-stage model for cancer risk assessment. I. The hazard function and the probability of tumor. Risk Anal; 8: 383–92.

Overton JH, Kimbell JS, Miller FJ. (2001) Dosimetry modeling of inhaled formaldehyde: the human respiratory tract. Toxicol Sci; 64: 122–34.

Peddada S, Dinse G, Kissling G. (2007) Incorporating historical control data when comparing tumor incidence rates. J Am Stat Assoc; 102: 1212–20.

Rao GN, Piegorsch WW, Haseman JK. (1987) Influence of body weight on the incidence of spontaneous neoplasms in rats and mice of long-term studies. Am J Clin Nutr; 45: 252–60.

Schulte-Hermann R, Bursch W, Marian B *et al*. (1999) Active cell death (apoptosis) and cellular proliferation as indicators of exposure to carcinogens. IARC Sci Publ; 146: 273–85.

SEER. (2005) Surveillance, epidemiology, and end results (SEER) program (www.seer.cancer.gov) SEER*Stat database: incidence—SEER 13 regs public-use, Nov 2004 sub for expanded races (1992–2002), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2005, based on the November 2004 submission. Bethesda, MD: National Cancer Institute.

Speit G, Merk O. (2002) Evaluation of mutagenic effects of formaldehyde *in vitro*: detection of crosslinks and mutations in mouse lymphoma cells. Mutagenesis; 17: 183–7.

Subramaniam RP, Richardson RB, Morgan KT *et al*. (1998) Computational fluid dynamics simulations of inspiratory airflow in the human nose and nasopharynx. Inhal Toxicol; 10: 92–120.

Subramaniam R, Chen C, Crump K *et al*. (2007) Uncertainties in the CIIT 2-stage model for formaldehyde-induced nasal cancer in the F344 rat: a limited sensitivity analysis—I. Risk Anal; 27: 1237–54.

Subramaniam R, Chen C, Crump K *et al*. (2008) Uncertainties in biologically-based modeling of formaldehyde-induced cancer risk: identification of key issues. Risk Anal; Accessed 18 June 2008, doi: 10.1111/j.1539-6924.2008.01083.x.

Tyihak E, Bocsi J, Timar F *et al*. (2001) Formaldehyde promotes and inhibits the proliferation of cultured tumour and endothelial cells. Cell Prolif; 34: 135–41.

USEPA. (2005) Guidelines for carcinogen risk assessment. Washington, DC: United States Environmental Protection Agency EPA/630/P-03/001F.

USEPA. (2006a) Health effects information used in cancer and noncancer risk characterization for the 1999 National-Scale Assessment (NATA). Available at http://www.epa.gov/ttn/atw/nata1999/99pdfs/healtheffectsinfo.pdf. Accessed 23 May 2008.

USEPA. (2006b) National Emission Standards for Hazardous Air Pollutants: Plywood and Composite Wood Products; List of Hazardous Air Pollutants, Lesser Quantity Designations, Source Category List; Final Rule. 40 CFR Part 63. Federal Reg; 71: 8341–8387.