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Dose Response for Formaldehyde-Induced Cytotoxicity in the Human Respiratory Tract

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Human studies of the sensory irritant effects of formaldehyde are complicated by the subjective nature of some clinical endpoints. This limits the usefulness of such studies for quantitative noncancer risk assessment of airborne formaldehyde. Objective measures of the noncancer effects of formaldehyde, such as the rate of regenerative cellular proliferation (RCP) secondary to cytolethality, can be obtained from laboratory animals but present the challenge of interspecies extrapolation of the data. To the extent that uncertainties associated with this extrapolation can be reduced, however, dose–response data obtained in laboratory animals are a viable alternative to clinical studies. Here, we describe the extrapolation of dose–response data for RCP from F344 rats to humans. Rats inhaled formaldehyde (0, 0.7, 2.0, 6.0, 10, and 15 ppm) 6 h/day, 5 days/week for up to 2 years. The dose response for RCP was J-shaped, with the rates of RCP at 0.7 and 2.0 ppm below but not statistically different from control, while the rates at the higher concentrations were significantly greater than control. Both the raw J-shaped data and a hockey-stick-shaped curve fitted to the raw data were used for predicting the human dose response for RCP. Cells lining the nasal airways of F344 rats and rhesus monkeys are comparably sensitive to the cytolethal effects of inhaled formaldehyde, suggesting that the equivalent human cells are also likely to be comparably sensitive. Using this assumption, the challenge of rat-to-human extrapolation was reduced to accurate prediction of site-specific flux of formaldehyde from inhaled air into the tissue lining the human respiratory tract. A computational fluid dynamics model of air flow and gas transport in the human nasal airways was linked to a typical path model of the human lung to provide site-specific flux predictions throughout the respiratory tract. Since breathing rate affects formaldehyde dosimetry, cytotoxicity dose–response curves were predicted for three standard working levels. With the most vigorous working level, the lowest concentrations of formaldehyde predicted to exert any cytotoxic effects in humans were 1.0 and 0.6 ppm, for the J-shaped and hockey-stick-shaped RCP curves, respectively. The predicted levels of response at the lowest effect concentrations are smaller than can be measured clinically. Published literature showing that the cytotoxicity of inhaled formaldehyde is related to exposure level rather than to duration of exposure suggests that the present analysis is a reasonable basis for derivation of standards for continuous human exposure.

***Key Words*: formaldehyde; dose response; cytotoxicity; regenerative cellular proliferation; dosimetry; CFD; computational fluid dynamics; nasal; working level.**

INTRODUCTION

Short-term consequences of formaldehyde inhalation include sensory irritation, the formation of DNA–protein cross-links (DPX), and cytotoxicity. Both sensory irritation and cytotoxicity are potential endpoints for use in noncancer risk assessments, while DPX are not adverse effects per se. Moreover, no data are available that clearly indicate the nature of the relationship, if any, between DPX formation and either cytotoxicity or sensory irritation. DPX data are not, therefore, suitable for a noncancer risk assessment for formaldehyde. Data on sensory irritation have the advantage of being collected in humans, but the data set on sensory irritation in humans is variable and subjective (Bender, 2001), which limits its usefulness for risk assessment. An extensive dose–response data set for regenerative cellular proliferation (RCP) secondary to formaldehyde-induced cytotoxicity in the F344 rat is available (Monticello, 1990; Monticello *et al.*, 1996) as is a human model that translates the inhaled concentration of formaldehyde into predictions of site-specific flux into tissue lining the respiratory tract (Overton *et al.*, 2001; Kimbell *et al.*, 2001a). By combining the RCP data from the rat with a human flux model, predictions of the extent and

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intensity of the cytotoxic response throughout the human respiratory tract were obtained. Because this approach is based on computer modeling, levels of response can be predicted that are below experimentally or clinically detectable levels. In the following, this modeling approach was used to predict the human dose response for cytotoxicity as the basis for a noncancer risk assessment for formaldehyde.

METHODS

Site-Specific Flux of Formaldehyde into Tissue

For a given inhaled concentration, the flux of formaldehyde from air into tissue lining the respiratory tract varies in a site-specific manner. This variation arises from several factors. The complicated shapes of the upper airways, particularly of the nasal passages, partition the inhaled air into distinct airstreams (Swift and Proctor, 1977; Girardin *et al.*, 1983; Subramaniam *et al.*, 1998). Cells lining the portions of airway surface over which these air streams flow experience greater exposures to airborne gasses and vapors than cells in other locations away from the major airstreams. Formaldehyde is reactive and soluble in the mucous that lines much of the upper respiratory tract, leading to rapid movement from air into the tissue. The formation of distinct airstreams combined with the propensity of formaldehyde to move from air into the tissue results in highly uneven site-specific tissue dosimetry for a given inhaled concentration. Accurate prediction of this site-specific dosimetry is essential to meaningful quantitative assessment of the relationship between inhaled concentration and cytolethality/RCP in tissue lining the respiratory tract, since the relationship between delivered dose and cytolethality/RCP is highly nonlinear. This predictive capability was obtained by combining two existing models. The computational fluid dynamics (CFD) model of Subramaniam *et al.* (1998) describes the human nasal passages in three dimensions while the typical path model of Overton and Graham (1995) uses a one-dimensional description of the entire human respiratory tract. These models were combined, taking advantage of the three-dimensional description of the nasal airways from the CFD model and using the typical path model for the remainder of the respiratory tract (Kimbell *et al.*, 2001b; Overton *et al.*, 2001). The hybrid CFD–typical path model provided the required capability for predicting site-specific flux throughout the human respiratory tract.

The hybrid model was used to partition the surface of the respiratory tract into discrete regions and to provide specific flux predictions for each of these regions. For the nose, this partitioning was based on model-predicted flux, with the entire flux range for the nasal airway lining tissue being divided into 20 equal segments or "flux bins". Note that this approach to defining flux bins means that a given bin could be physically discontinuous across the nasal epithelium. We found that the level of anatomical resolution provided by 20 flux bins was sufficient to capture the major effects of airway anatomy on regional dosimetry in the nose (Kimbell *et al.*, 2001b). For the remainder of the respiratory tract, the partitioning strategy was based on anatomical structure rather than on predicted flux (Overton *et al.*, 2001). A total of 25 flux bins were used to describe the lower respiratory tract. The sequentially branching structure of the lower airways and the tendency of formaldehyde to move rapidly into the lining tissue causes the progressively more distal bins to have less and less flux for a given inhaled concentration.

Working Levels

The hybrid dosimetry model predicts different site-specific patterns of flux into tissue depending on the rate and route of breathing, assuming continuous exposure to a specific concentration of formaldehyde. To determine how differences in activity levels could affect the predicted dose-response for cytotoxicity, minute ventilation for each of three exertion levels ("working levels") was specified according to standard values promulgated by the International Commission on Radiological Protection (Snipes *et al.*, 1997; Table 1). These working levels describe the minute ventilation (i.e., ventilatory drive) for each hour of a day. The site-specific

TABLE 1
Working Levels

Working level classification	Time (h) per day spent at activity level			
	Sleeping (7.5 liters/min)[a]	Sitting (9.0 liters/min)[a]	Light activity (25 liters/min)[a]	Heavy activity (50 liters/min)[a,b]
(1) Day not at work	8	8	8	—
(2) Light working	8	6	9	1
(3) Heavy working	8	4	10	2

[a] Minute ventilation.
[b] Heavy activity breathing rate is oronasal. All other breathing rates are nasal only.

flux of formaldehyde into tissue was calculated for each working level as follows. For working level classification 1, the time-weighted average flux in flux bin i is given by

$$Flux_{i,1} = \frac{A_i + B_i + C_i}{3}, \quad (1)$$

where A_i, B_i, and C_i are the model-predicted fluxes for the minute ventilation levels of 7.5, 9.0, and 25.0 liters/min, respectively. Working level 1 describes a daily breathing pattern associated with a resting or very light activity level. For working level classifications 2 and 3, the calculations assume that 2 days a week are spent at working level 1 with the rest of the week spent at either working level 2 or working level 3, which correspond to higher levels of activity associated with physical labor or exercise, with level 3 being the most vigorous. For working level 2, the time-weighted average flux in bin i is given by

$$Flux_{i,2} = \left(\frac{5}{7}\right) \cdot \frac{8 \cdot A_i + 6 \cdot B_i + 9 \cdot C_i + 1 \cdot D_i}{24} + \left(\frac{2}{7}\right) \cdot \frac{A_i + B_i + C_i}{3}, \quad (2)$$

where A_i, B_i, C_i, and D_i are the bin-specific fluxes for the 7.5, 9.0, 25.0 and 50.0 liters/min breathing rates, respectively. For working level 3, the time-weighted average flux in bin i is given by

$$Flux_{i,3} = \left(\frac{5}{7}\right) \cdot \frac{8 \cdot A_i + 4 \cdot B_i + 10 \cdot C_i + 2 \cdot D_i}{24} + \left(\frac{2}{7}\right) \cdot \frac{A_i + B_i + C_i}{3}. \quad (3)$$

Flux-Cytotoxicity Dose Response

Monticello *et al.* (1996) exposed rats for 6 h/day, 5 days/week for varying periods of time. The rate of formaldehyde-stimulated RCP was measured at seven sites in the F344 rat nose at 1, 4, and 10 days and at 6, 13, 26, 52, and 78 weeks. The present analysis used data from the five sites (anterior lateral meatus, posterior lateral meatus, anterior medial septum, posterior medial septum, and medial maxilloturbinate) with the most complete data sets (Fig. 1). RCP was seen at 6, 10, and 15 ppm but not at 0.7 or 2 ppm. We assumed for this work that RCP is a direct consequence of the cytolethal effects of formaldehyde.

Extrapolation of the rat RCP data for use in the human model required several adjustments of the data. First, for a given exposure level, the relationship between duration of exposure and intensity of RCP varied with time (Fig. 1). The most intense responses tended to occur during the first 6 weeks of exposure. Since it was not clear how the data on temporal variation in RCP in the rat should be extrapolated to the human model, a time-weighted average RCP was calculated for the entire 78 weeks of exposure for each site at which RCP was measured. The calculation removed the explicit time dependence of the data and provided a single RCP value for each measured site and exposure concentration. Second, site-to-site variation in RCP did not vary with predicted flux in a consistent manner (data not shown), possibly due to variability in the RCP data. Sources of this variability include the squamous metaplasia that occurs in response to higher levels of formaldehyde exposure (Kimbell *et al.*, 1997) and experimental variability in the measurement of RCP. Misspecification of the CFD model itself is unlikely to have been the reason for the lack of correlation between site-specific predictions of flux and RCP. The air flow predictions of the CFD model agreed with measurements in a human nasal mold (Subramaniam *et al.*, 1998) and site-specific predictions of formaldehyde flux from air into tissue were shown in simulation studies to be consistent with respiratory mucosal DNA–protein cross-link levels after formaldehyde inhalation in F344 rats and rhesus monkeys (Conolly *et al.*, 2000). Consistent variation of RCP with predicted flux was obtained, however, by averaging RCP and predicted flux across the five sites (Table 2). The empirical function (table function) thereby obtained was used without further adjustment in the hybrid human dosimetry model. These data reduction steps leading to development of the table function allowed a straightforward extrapolation of the rat data for use in the human model. Use of the rat data in the human model without reduction would require assumptions about the mapping of the time dependence of the rat data to the human case and a better understanding of the sources of variability in site-specific measurements of RCP in the rat nose.

Although it was published later, the experimental work reported by Monticello *et al.* (1996) was actually completed before the development of the first CFD model of the rat anterior nasal airways (Kimbell *et al.*, 1993) that provides high-resolution, site-specific predictions of formaldehyde flux from air into the tissue. These predictions indicate that the site of highest flux in the rat nose is not one of the six sites that were sampled experimentally by Monticello *et al.* (1996). For a given cytolethal exposure to formaldehyde, this site of highest flux would be expected to have a higher rate of RCP than any of the six sampled sites. In a recent cancer risk assessment for formaldehyde (CIIT, 1999) the maximum rate of RCP in the nose was defined as an adjustable parameter (α_{max}). The value of α_{max} was estimated statistically by optimizing the fit of the cancer risk model to rat tumor data at the 14.96 ppm, the highest exposure concentration used by Monticello *et al.* (1996) (Table 2).

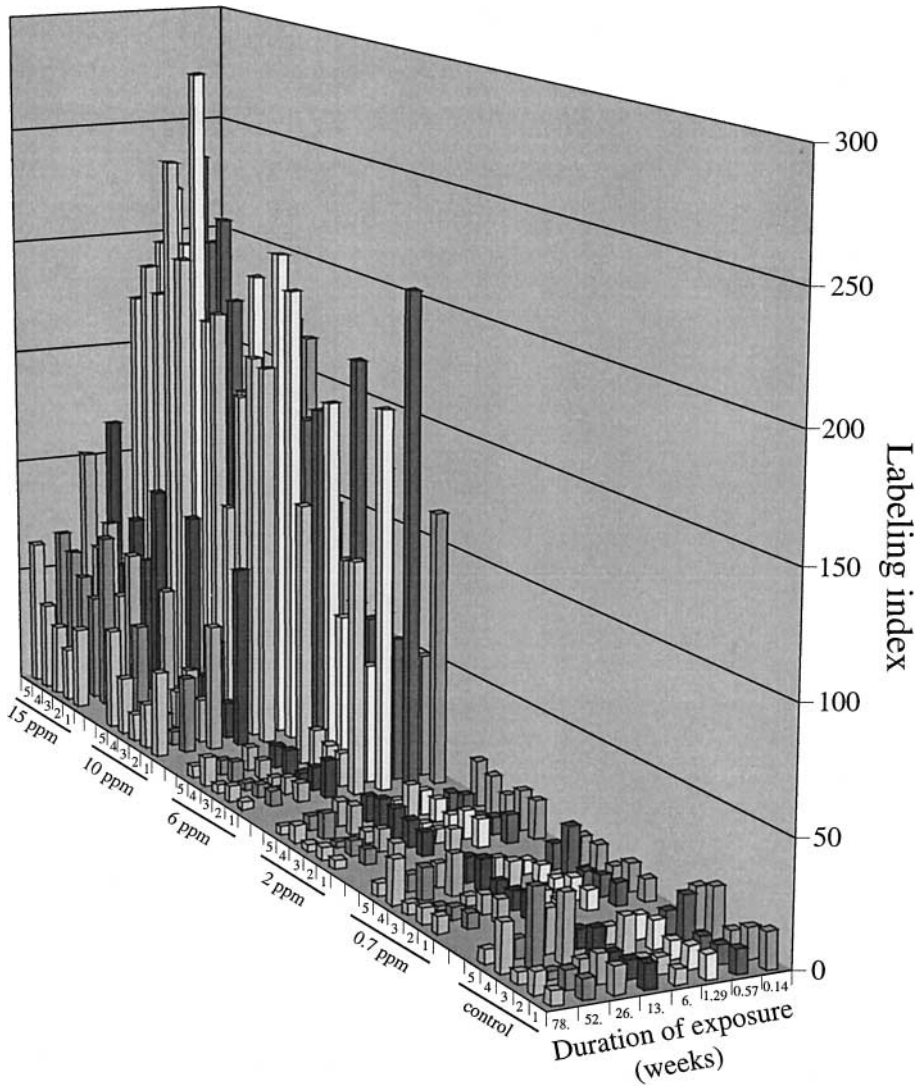

FIG. 1. Labeling index data from F344 rats exposed by inhalation to various concentrations of formaldehyde (Monticello *et al.*, 1996). Exposures were 6 h/day, 5 days/week, with exposure durations from 1 day (0.14 weeks) to 78 weeks. Labeling index was measured at specific sites in the nasal epithelium. Data are presented here for the anterior lateral meatus, posterior lateral meatus, anterior medial septum, posterior medial septum, and medial maxilloturbinate. These sites correspond to numerals 1–5 for a given exposure concentration. Labeling index for exposure durations from 1 day through 6 weeks was measured by injecting BrdU while subsequent time points used osmotic minipumps implanted for 3 days. The injection data were transformed to equivalent minipump data using a factor of 6.83, which was the ratio of minipump labeling index to injection labeling index for all of the control data.

Finally, the dose–response data measured by Monticello *et al.* (1996) was J-shaped (Table 2). Rates of RCP measured at 0.7 and 2.0 ppm were slightly below the control rate but were not statistically lower ($P > 0.05$). The rates measured at 6, 10, and 15 ppm were significantly greater than control. Since the J-shaped data were not statistically significantly different from a hockey-stick-shaped (threshold) dose–response curve, we used both the J-shaped data and a hockey-stick-shaped model for dose–response modeling. Two alternative approaches were evaluated for estimating the parameters of the hockey stick model. First, an isotonic regression model (Gaines and Rice, 1990) was fit to the RCP data. For this approach, the inflection point of the hockey stick must be one of the experimental concentrations and was set to 2 ppm. With this model there are three parameters: β_{01} (intercept at ppm = 0), β_{02} (intercept for positive-slope line) and β_{12} (positive slope). The averaging methods of Williams (1971, 1972) were used in order to obtain a monotonic nondecreasing relationship between RCP values and formaldehyde exposure concentration. Second, a two-segment model was used where the inflection point is estimated as part of the model fitting process. The two-segment model has the

TABLE 2
Formaldehyde Flux and Cell Division Rate Constants for Rats

Formaldehyde (ppm)	Average flux across five sampled sites in rat nose[a] (pmol/mm^2/h)	(J-shaped) Average division rate constant (RCP) for cells in the five sites (1/h)	(Hockey-stick-shaped[b]) Average division rate constant (RCP) for cells in the five sites (1/h)
0	0.00×10^0	3.39×10^{-4}	2.79×10^{-4}
0.7	4.36×10^2	2.59×10^{-4}	2.79×10^{-4}
2.0	1.24×10^3	2.41×10^{-4}	2.79×10^{-4}
6.01	3.74×10^3	4.86×10^{-4}	4.86×10^{-4}
9.93	6.23×10^3	1.62×10^{-3}	1.62×10^{-3}
14.96	9.34×10^3	3.06×10^{-3}	3.06×10^{-3}
14.96	$(3.93 \times 10^4)^c$	$(2.0 \times 10^{-2})^d$	$(2.0 \times 10^{-2})^d$

[a] Kimbell *et al.* (2001a).
[b] Hockey-stick-shaped model fit to J-shaped dose–response data as described under Methods.
[c] Highest flux at any site predicted by CFD model at 14.96 ppm.
[d] α_{max},the division rate corresponding to highest flux. Estimated by maximizing the fit of a rat clonal growth model to nasal squamous cell carcinoma data (CIIT, 1999).

form

$$RCP = \left(\frac{\beta_{01}}{\beta_{02} + \beta_{12} * ppm} \; \frac{ppm < ppm_{\text{inf}}}{ppm \geq ppm_{\text{inf}}} \right), \quad (4)$$

where the inflection point in the two-segment model, ppm_{inf}, is given by

$$ppm_{\text{inf}} = \frac{\beta_{01} - \beta_{02}}{\beta_{12}}. \quad (5)$$

Confidence intervals for the two-segment model were based on standard error propagation rules and the estimated standard errors and covariances for the model parameters.

The dose response for four parameters of cytotoxicity was predicted:

1. The percentage of the respiratory tract surface area (%surface) for which the rate of cell division was greater than control.
2. The percentage of the nasal surface area (%surface_N) for which the rate of cell division was greater than control.
3. The intensity of the cytotoxic response:

$$Rindex = \begin{pmatrix} \sum_{n=1}^{N} SA_{\text{bin}\,n} \cdot (\alpha_f - \alpha_b) & \text{if } \alpha_f > \alpha_c \\ 0 & \text{otherwise} \end{pmatrix}, \quad (6)$$

where N is the total number of flux bins covering the entire surface of the respiratory tract (45 in this analysis) and $SA_{\text{bin}\,n}$ is the surface area in flux bin n, while α_f and α_c are division rate constants for formaldehyde exposure and controls, respectively. By including information on the magnitude of the effect of formaldehyde on the rate of cell replication, Rindex is intended to provide an indication of the intensity of the cytotoxic response. While the surface area parameters %surface and %surface_N can reach maximum values (i.e., no more than 100% of the surface can be involved), the value of Rindex is not similarly bounded and can continue to increase with increasing levels of exposure. Rindex thus provides information on how the cytotoxic response is changing with increasing exposure even when the values of %surface and %surface_N may not be changing.

4. Rindex_N describes the intensity of the cytotoxic response in the nose. The formulation of Rindex_N is given by Eq. (1) except that the flux bins are limited to those lining the nasal airways.

Implementation of the Model

The computer model for prediction of cytotoxicity dose response was written in the continuous simulation language ACSL (The AEgis Technologies Group, Inc., Huntsville, AL). A copy of the code may be requested by sending e-mail to R.B.C (rconolly@ciit.org). Simulations were run on 200- to 400-mHz Pentium computers with individual runs taking no more than a few seconds.

RESULTS

Hockey-Stick-Shaped Model for RCP Dose Response

Isotonic regression and two-segment models were used to derive alternative hockey-stick-shaped dose–response curves for RCP from the raw J-shaped data. With the inflection point fixed at 2 ppm, fitting the isotonic regression model to the RCP data provided a root

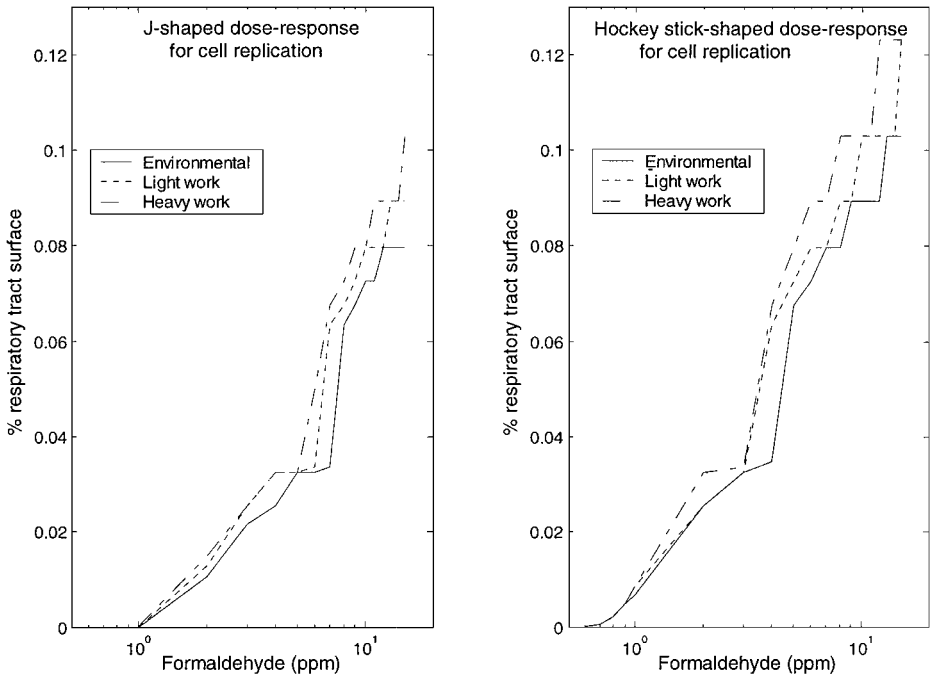

FIG. 2. Model-predicted dose-response for percentage of respiratory tract surface area affected by cytotoxicity (parameter %surface). Simulation shown in the left panel used the J-shaped dose response for cytotoxicity while that in the right panel used the hockey-stick-shaped dose response. In the legend, "environmental", "light", and "heavy" refer to working levels 1, 2, and 3, respectively. The jagged shape of the dose–response curves results from the partitioning of the respiratory tract surface area into flux bins (see Methods).

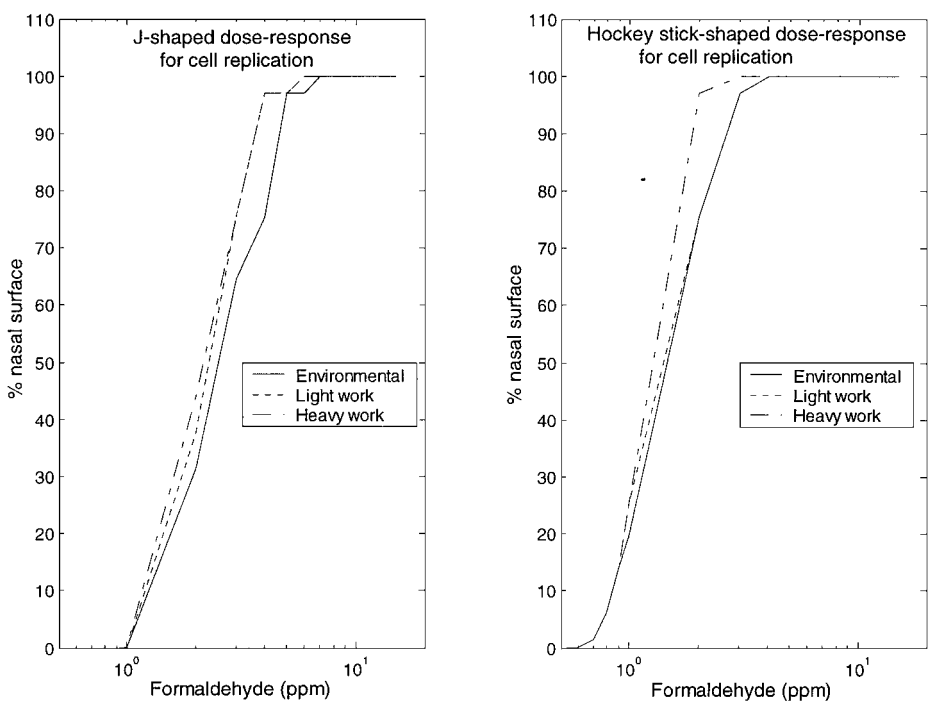

FIG. 3. Model-predicted dose response for percentage of the nasal surface area affected by cytotoxicity (parameter %surface_N). The jagged shape of the dose–response curves results from the partitioning of the respiratory tract surface area into flux bins (see Methods). Other details as for Fig. 2.

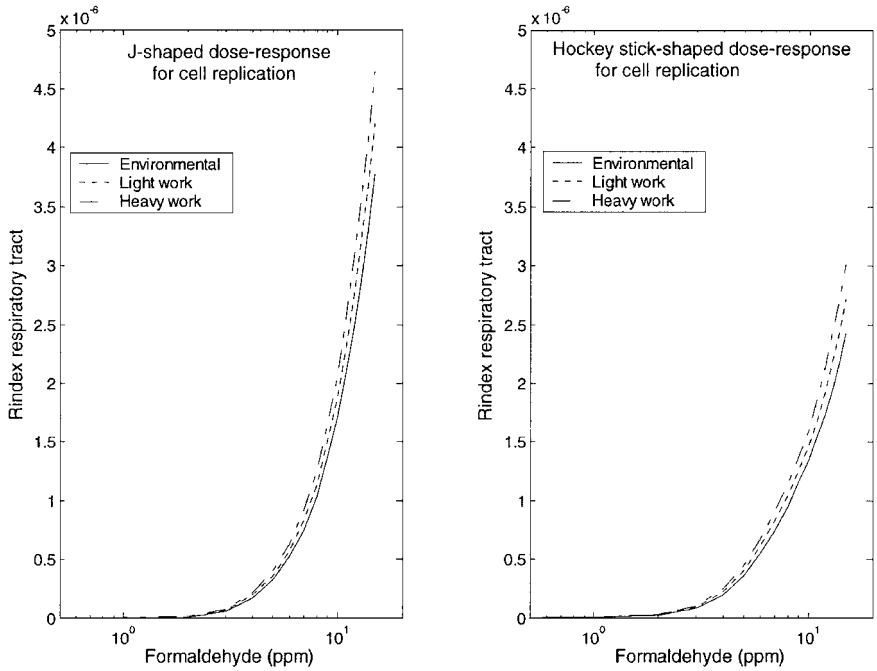

FIG. 4. Model-predicted dose response for parameter Rindex. Other details as for Fig. 2.

mean square error of 5.91 based on 4 degrees of freedom. Use of the two-segment model allowed the data to specify the location of the inflection point, which was 4.8 with asymmetric confidence interval (3.74, 5.82). The root mean square error for the two-segment model was 2.35, based on 3 degrees of freedom. The two-segment model thus provided a better fit to the RCP data better than the isotonic regression model. However, we chose to use the hockey-stick-shaped curve obtained with the isotonic regression model for modeling cytotoxicity dose

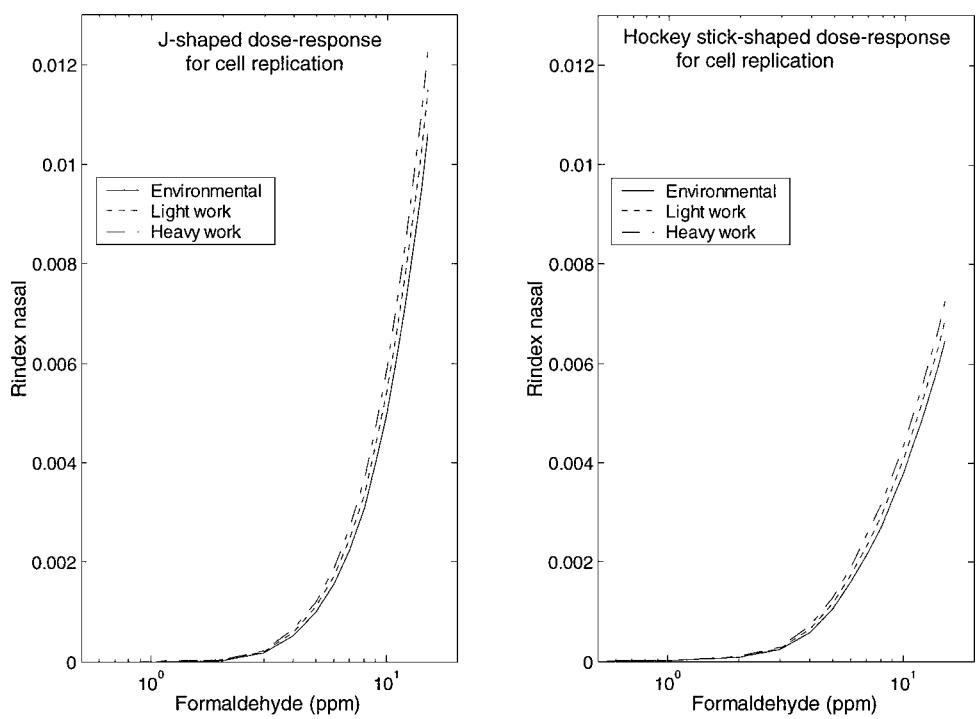

FIG. 5. Model-predicted dose response for the parameter Rindex_N. Other details as for Fig. 2.

TABLE 3
Working Level 1: Hockey-Stick-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤0.5	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.6	0.1245E−04	0.3702E−01	0.8937E−12	0.2657E−08
0.7	0.5159E−03	0.1534E+01	0.2523E−10	0.7504E−07
0.8	0.2096E−02	0.6232E+01	0.2567E−09	0.7634E−06
0.9	0.4766E−02	0.1417E+02	0.7769E−09	0.2310E−05
1.0	0.6586E−02	0.1958E+02	0.1692E−08	0.5030E−05
2.0	0.2537E−01	0.7545E+02	0.2528E−07	0.7517E−04
3.0	0.3261E−01	0.9696E+02	0.8103E−07	0.2410E−03
4.0	0.3473E−01	0.1000E+03	0.1971E−06	0.5860E−03
5.0	0.6748E−01	0.1000E+03	0.3633E−06	0.1058E−02
6.0	0.7263E−01	0.1000E+03	0.5526E−06	0.1588E−02
7.0	0.7963E−01	0.1000E+03	0.7459E−06	0.2128E−02
8.0	0.7963E−01	0.1000E+03	0.9532E−06	0.2706E−02
9.0	0.8938E−01	0.1000E+03	0.1155E−05	0.3266E−02
10.0	0.8938E−01	0.1000E+03	0.1353E−05	0.3814E−02
11.0	0.8938E−01	0.1000E+03	0.1547E−05	0.4346E−02
12.0	0.8938E−01	0.1000E+03	0.1740E−05	0.4879E−02
13.0	0.1030E+00	0.1000E+03	0.1951E−05	0.5414E−02
14.0	0.1030E+00	0.1000E+03	0.2186E−05	0.5939E−02
15.0	0.1030E+00	0.1000E+03	0.2426E−05	0.6461E−02

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

response (Table 2). This choice reflected the desire to be conservative with respect to risk estimation when faced with choices that were not clearly determined by the data.

Simulations of Cytotoxicity Dose Response

Dose–response simulations for %surface, %surface_N, Rindex, and Rindex_N are shown in Figs. 2–5, respectively. The numerical simulation results on which these plots are based are provided in Tables 3–8.

Using the J-shaped curve for RCP, the predicted lowest effect concentration was 1 ppm at working level 3 (Table 8). For both working levels 1 and 2 the predicted lowest effect concentration was 2 ppm (Tables 4 and 6).

The much smaller predicted Rindex and Rindex_N responses at 1–2 ppm, relative to the responses predicted at 10–15 ppm (e.g., Table 8) indicate that the cytotoxic responses predicted for 1–2 ppm are relatively mild (i.e., the increase in the rate of cell replication above the control level is small).

Using the hockey-stick-shaped curve for RCP, the predicted lowest effect concentration was 0.6 ppm

TABLE 4
Working Level 1: J-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤1.0	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
2.0	0.1054E−01	0.3135E+02	0.6948E−08	0.2066E−04
3.0	0.2168E−01	0.6446E+02	0.5698E−07	0.1694E−03
4.0	0.2537E−01	0.7545E+02	0.1705E−06	0.5070E−03
5.0	0.3261E−01	0.9696E+02	0.3297E−06	0.9805E−03
6.0	0.3261E−01	0.9696E+02	0.5228E−06	0.1555E−02
7.0	0.3363E−01	0.1000E+03	0.7489E−06	0.2227E−02
8.0	0.6364E−01	0.1000E+03	0.1032E−05	0.3056E−02
9.0	0.6748E−01	0.1000E+03	0.1356E−05	0.3985E−02
10.0	0.7263E−01	0.1000E+03	0.1706E−05	0.4988E−02
11.0	0.7263E−01	0.1000E+03	0.2080E−05	0.6061E−02
12.0	0.7963E−01	0.1000E+03	0.2465E−05	0.7163E−02
13.0	0.7963E−01	0.1000E+03	0.2873E−05	0.8289E−02
14.0	0.7963E−01	0.1000E+03	0.3322E−05	0.9454E−02
15.0	0.7963E−01	0.1000E+03	0.3778E−05	0.1063E−01

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

TABLE 5
Working Level 2: Hockey-Stick-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤0.5	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.6	0.1245E−04	0.3702E−01	0.1099E−11	0.3268E−08
0.7	0.5159E−03	0.1534E+01	0.3471E−10	0.1032E−06
0.8	0.2096E−02	0.6232E+01	0.3055E−09	0.9083E−06
0.9	0.4766E−02	0.1417E+02	0.8946E−09	0.2660E−05
1.0	0.8526E−02	0.2535E+02	0.1922E−08	0.5716E−05
2.0	0.2537E−01	0.7545E+02	0.2776E−07	0.8255E−04
3.0	0.3261E−01	0.9696E+02	0.8930E−07	0.2655E−03
4.0	0.6364E−01	0.1000E+03	0.2220E−06	0.6513E−03
5.0	0.7263E−01	0.1000E+03	0.4060E−06	0.1162E−02
6.0	0.7963E−01	0.1000E+03	0.6103E−06	0.1729E−02
7.0	0.7963E−01	0.1000E+03	0.8288E−06	0.2334E−02
8.0	0.8938E−01	0.1000E+03	0.1050E−05	0.2942E−02
9.0	0.8938E−01	0.1000E+03	0.1264E−05	0.3530E−02
10.0	0.1030E+00	0.1000E+03	0.1472E−05	0.4097E−02
11.0	0.1030E+00	0.1000E+03	0.1682E−05	0.4661E−02
12.0	0.1030E+00	0.1000E+03	0.1930E−05	0.5225E−02
13.0	0.1030E+00	0.1000E+03	0.2188E−05	0.5776E−02
14.0	0.1030E+00	0.1000E+03	0.2448E−05	0.6326E−02
15.0	0.1226E+00	0.1000E+03	0.2715E−05	0.6876E−02

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

TABLE 6
Working Level 2: J-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤0.1	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.2	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.3	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.4	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.5	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.6	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.7	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.8	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.9	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
1.0	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
2.0	0.1268E−01	0.3769E+02	0.7902E−08	0.2350E−04
3.0	0.2537E−01	0.7545E+02	0.6400E−07	0.1903E−03
4.0	0.3261E−01	0.9696E+02	0.1920E−06	0.5709E−03
5.0	0.3261E−01	0.9696E+02	0.3652E−06	0.1086E−02
6.0	0.3363E−01	0.1000E+03	0.5731E−06	0.1704E−02
7.0	0.6364E−01	0.1000E+03	0.8310E−06	0.2459E−02
8.0	0.6748E−01	0.1000E+03	0.1144E−05	0.3350E−02
9.0	0.7263E−01	0.1000E+03	0.1495E−05	0.4350E−02
10.0	0.7963E−01	0.1000E+03	0.1880E−05	0.5448E−02
11.0	0.7963E−01	0.1000E+03	0.2285E−05	0.6594E−02
12.0	0.7963E−01	0.1000E+03	0.2737E−05	0.7770E−02
13.0	0.8938E−01	0.1000E+03	0.3220E−05	0.8997E−02
14.0	0.8938E−01	0.1000E+03	0.3707E−05	0.1023E−01
15.0	0.8938E−01	0.1000E+03	0.4199E−05	0.1145E−01

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

(Tables 3, 5, and 7). All working levels were predicted to cause some response at this level.

Cytotoxicity is predicted to involve only a small fraction of the entire respiratory tract surface area, even at very high formaldehyde concentrations (Tables 3–8). This reflects the efficient scrubbing of formaldehyde from inhaled air into tissue in the nasal airways. The model predicts that toxicologically significant doses of formaldehyde do not reach the lower respiratory tract, even at high inhaled concentrations. This prediction is consistent with the site specificity of the acute and chronic effects of inhaled formaldehyde. With the analysis restricted to the nasal region, however, the model predicts larger fractions of affected airway surface area, though only at relatively high concentrations (Fig. 3). For example, 100% involvement of the nasal lining epithelium is predicted for 3 ppm at working level 3 using the hockey-stick-shaped RCP curve and for 7 ppm at working level 1 using the J-shaped curve. It should be noted though that these concentrations are well above the formaldehyde exposure levels normally encountered in either environmental or occupational settings.

TABLE 7
Working Level 3: Hockey-Stick-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤0.5	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
0.6	0.1245E−04	0.3702E−01	0.1288E−11	0.3830E−08
0.7	0.5159E−03	0.1534E+01	0.4356E−10	0.1295E−06
0.8	0.2096E−02	0.6232E+01	0.3514E−09	0.1045E−05
0.9	0.4766E−02	0.1417E+02	0.1006E−08	0.2991E−05
1.0	0.8526E−02	0.2535E+02	0.2160E−08	0.6424E−05
2.0	0.3261E−01	0.9696E+02	0.3052E−07	0.9075E−04
3.0	0.3363E−01	0.1000E+03	0.9760E−07	0.2902E−03
4.0	0.6748E−01	0.1000E+03	0.2497E−06	0.7172E−03
5.0	0.7963E−01	0.1000E+03	0.4521E−06	0.1274E−02
6.0	0.8938E−01	0.1000E+03	0.6735E−06	0.1882E−02
7.0	0.8938E−01	0.1000E+03	0.9136E−06	0.2540E−02
8.0	0.1030E+00	0.1000E+03	0.1146E−05	0.3175E−02
9.0	0.1030E+00	0.1000E+03	0.1372E−05	0.3784E−02
10.0	0.1030E+00	0.1000E+03	0.1597E−05	0.4378E−02
11.0	0.1030E+00	0.1000E+03	0.1872E−05	0.4970E−02
12.0	0.1226E+00	0.1000E+03	0.2151E−05	0.5549E−02
13.0	0.1226E+00	0.1000E+03	0.2438E−05	0.6127E−02
14.0	0.1226E+00	0.1000E+03	0.2725E−05	0.6704E−02
15.0	0.1226E+00	0.1000E+03	0.3016E−05	0.7270E−02

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

TABLE 8
Working Level 3: J-Shaped Replication

ppm	%Surface[a]	%Surface_N[b]	Rindex[c]	Rindex_N[d]
≤0.9	0.0000E+00	0.0000E+00	0.0000E+00	0.0000E+00
1.0	0.1245E−04	0.3702E−01	0.8789E−13	0.2614E−09
2.0	0.1475E−01	0.4386E+02	0.8905E−08	0.2648E−04
3.0	0.2537E−01	0.7545E+02	0.7149E−07	0.2126E−03
4.0	0.3261E−01	0.9696E+02	0.2146E−06	0.6380E−03
5.0	0.3261E−01	0.9696E+02	0.4031E−06	0.1199E−02
6.0	0.4995E−01	0.1000E+03	0.6283E−06	0.1866E−02
7.0	0.6748E−01	0.1000E+03	0.9207E−06	0.2693E−02
8.0	0.7263E−01	0.1000E+03	0.1259E−05	0.3647E−02
9.0	0.7963E−01	0.1000E+03	0.1642E−05	0.4731E−02
10.0	0.7963E−01	0.1000E+03	0.2061E−05	0.5910E−02
11.0	0.8938E−01	0.1000E+03	0.2547E−05	0.7136E−02
12.0	0.8938E−01	0.1000E+03	0.3058E−05	0.8412E−02
13.0	0.8938E−01	0.1000E+03	0.3578E−05	0.9695E−02
14.0	0.8938E−01	0.1000E+03	0.4099E−05	0.1098E−01
15.0	0.1030E+00	0.1000E+03	0.4646E−05	0.1231E−01

[a] Percentage of respiratory tract surface area for which the rate of cell division was predicted to be greater than control.
[b] Percentage of nasal surface area for which the rate of cell division was predicted to be greater than control.
[c] Sum over the flux bins lining the entire respiratory tract of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.
[d] Sum over the flux bins lining the nasal airways of the product of the difference between the actual and basal rates of cell division for each flux bin and the surface area of that flux bin.

DISCUSSION

The lowest concentration at which formaldehyde is predicted to exert any cytotoxic effects is 1.0 ppm using the J-shaped replication curve and 0.6 ppm using the hockey-stick-shaped replication curve. The modeling suggests that the choice of the shape of the dose–response curve for regenerative cellular proliferation (J- or hockey-stick-shaped) is more important than the working level, though small effects of working level are seen. It should be noted that the J-shaped curve is based on the original data, while the hockey-stick-shaped curve was obtained fitting a statistical model to the original data.

This analysis used RCP data from rats as a surrogate for human data. The major uncertainties associated with this use of the rodent data are: (1) whether or not the equivalent human cells are similarly sensitive to the cytotoxic effects of formaldehyde; and (2) how the cytotoxicity data, which were obtained using a 6 h/day, 5 days/week exposure regimen, should be used for the derivation of human exposure standards for different exposure patterns.

While the mechanism by which formaldehyde exerts its cytotoxic effects is not known, formaldehyde reacts directly with tissue constituents, and cytotoxicity is presumably a function of this reactivity. A key observation is that the formaldehyde flux-regenerative cellular replication relationship in F344 rats and rhesus monkeys is similar (Kimbell *et al.*, 2001a). This suggests that rodent–primate differences in susceptibility to the cytotoxic effects of formaldehyde are small and increase confidence in the use of the rat data for human dose–response modeling.

Monticello *et al.* (1996) described the dose–response for RCP in the rat nasal epithelium (Fig. 1). All the exposures for that work were 6 h/day. Use of these data to estimate acceptable continuous levels of exposure to formaldehyde requires calculation of a human equivalent concentration (HEC) for continuous exposure. The default approach used by the US EPA, for the case of continuous exposure, would be to assume a constant $C \times T$ (concentration $\times$ time) relationship. This means that, for example, the level of toxicity associated with exposure to 6 ppm formaldehyde for 6 h (36 ppm-h) would be assumed to be the same as exposure to 2 ppm for 18 h. For the formaldehyde data described by Monticello *et al.* (1996) the default, constant $C \times T$ adjustment would use factors of 6/24 and 5/7 to account for the difference in exposure regimen (i.e., HEC for continuous exposure $= \text{ppm}_{\text{rat}} \times 6/24 \times 5/7$).

Several studies have provided data relevant to the $C \times T$ issue for formaldehyde. Rusch *et al.* (1983) exposed cynomologus monkeys, F344 rats, and Syrian golden hamsters to 0, 0.19, 0.98, and 2.95 ppm formaldehyde 22 h/day, 7 days/week for 22 weeks. Adverse effects were seen in monkeys and rats at the highest but not at the other exposure levels. No adverse effects were seen in hamsters at any exposure level. The exposure regimen used by Rusch *et al.* provides 5.1 times more exposure in ppm-h per week than the regimen used by Monticello *et al.* (1996). The 0.98 and 2.95 exposure levels used by Rusch *et al.* thus correspond to 5.1 and 15 ppm, respectively for 6 h/day, 5 days/week, assuming constant $C \times T$. Rusch *et al.* saw mild cytotoxic effects in the F344 rat at 2.98 ppm, in contrast to the severe cytotoxicity seen by Monticello *et al.* at 15 ppm. The lack of effect seen at 0.98 ppm by Rusch *et al.* is consistent with the lack of response seen by Monticello *et al.* at 6 ppm for time points later than 6 weeks.

Swenberg *et al.* (1985) examined the $C \times T$ relationship in rats that inhaled formaldehyde. A constant $C \times T$ relationship was seen in an anterior region of the rat nose with little or no mucociliary clearance but not in a more posterior region having significant mucociliary clearance. For the anterior region in which a constant $C \times T$ relationship was seen, exposures of 3 ppm for 12 h, 6 ppm for 6 h, and 12 ppm for 3 h were similarly toxic, as evaluated by increases in labeling index. For the more posterior region of the rat nose that departed from a constant $C \times T$ relationship, toxicity was a stronger function of exposure concentration than of duration (12 ppm for 3 h was more toxic than 3 ppm for 12 h). Given prevalence of mucocilliary clearance in the rat nose, these data suggest that departures from constant $C \times T$ relationships are the norm.

Wilmer *et al.* (1987) exposed Wistar rats for 4 weeks, 5 days/week, to 0, 5, or 10 ppm formaldehyde continuously (8 h/day) or to 10 or 20 ppm intermittently (eight 30-min exposure periods separated by 30-min nonexposure periods in a day). Nasal epithelial cell toxicity as assessed by histopathology and labeling index correlated with exposure level (ppm) but not with total dose defined as ppm-h. Wilmer *et al.* (1989) used the same basic design but with exposure levels of 0, 1, 2, and 4 ppm and also found that toxicity correlated with exposure level but not with total dose.

While assumption of a constant $C \times T$ relationship can be appropriate as a conservative approach in the absence of other relevant information, available data suggest that $C \times T$ is not a constant under real-world conditions of exposure to formaldehyde. The laboratory animal inhalation toxicity studies reviewed briefly above (Rusch *et al.*, 1983; Swenberg *et al.*, 1985; Wilmer *et al.*, 1987, 1989) indicate that the cytotoxic effects of inhaled formaldehyde do not obey a constant $C \times T$ relationship. Monticello *et al.* (1996) show a J-shaped dose response for RCP in the F344 rat (Table 2). These data also show a lack of cytotoxicity and RCP at 2 ppm and below. Other studies (Magana-Schwencke and Moustacchi, 1980; Snyder and Van Houten, 1986; Permana and Snapka, 1994) have shown that formaldehyde inhibits DNA replication, and Heck and Casanova (1999) described a quantitative, biologically motivated model for

the arrest of DNA replication by DPX. To the extent that formaldehyde-induced cytotoxicity is due to DPX, as suggested by Heck and Casanova (1999), the fairly rapid removal of DPX (Casanova *et al.*, 1994) means that cytotoxicity dependent on accumulation of DPX would not occur until higher concentrations of inhaled formaldehyde at which the rate of DPX formation exceeds the rate of DPX removal and some critical level of DPX is achieved. The overall weight of this evidence, and of human experience, is that low concentrations of formaldehyde are not cytotoxic, regardless of duration of exposure. (These same concentrations may well reduce rates of cell division in exposed epithelium but this effect is presumably not clinically significant.) This weight of the evidence is not consistent with a constant $C \times T$ for formaldehyde-induced cytotoxicity (and RCP), which implies a toxic response at any exposure level given sufficient exposure duration. The inhibitory effect of formaldehyde on the rate of cell division does argue strongly that environmental levels of exposure to formaldehyde, which are typically in the range of a few ppb, do not pose a cytotoxic risk. $C \times T$ corrections would be inappropriate for these low levels of exposure.

Use of computer-based dosimetry models to predict regional flux in the human nose and throughout the human respiratory tract strengthens this analysis. This predictive capability combines a high-resolution reconstruction of a human nose with a robust model of the lung. The model predicts that cytotoxicity occurs in the rat at lower concentrations than were seen experimentally. This possibility arises because (1) the model-generated predictions are made for the entire surface of the respiratory tract, while experimental studies examined only a limited number of sites, and (2) the sensitivity of the modeling to predict low levels of response is limited only by the numerical precision of the software. The model was implemented in the computer using double precision arithmetic having 16 significant figures while laboratory measurement of RCP achieves 3 significant figures at best. The computer model can therefore predict effects that would not be observable experimentally. For example, the predicted mild cytotoxic response involving 0.03% (i.e., 3 mm^2 of every 10,000 mm^2) of the nasal surface area at 1.0 ppm (Table 7) would almost certainly not be clinically detectable. No data are available, however, that tell us what combination of percentage of surface area and intensity of response is clinically significant or might constitute an adverse effect.

Simulations were conducted for various working levels (Table 1), including a level associated with strenuous exercise (level 3). Predicted dose responses for working level 3 represent a worst case in the sense that at this working level considerably more formaldehyde is being inhaled than for the less strenuous, more typical working levels. This factor also contributes to the weight of evidence when defining HECs for exposure regimens different from 6 h/day, 5 days/week.

Though largely of academic interest, simulations of exposures up to 15 ppm formaldehyde show different dose–response behaviors for the parameters %surface and Rindex. A plateau in the dose response for %surface develops, while Rindex increase with increasing concentration. These behaviors can be interpreted in a straightforward manner. The model predicts that the ability of the nose and upper respiratory tract to scrub formaldehyde out of inhaled air protects the lower respiratory tract. Thus, even at very high inhaled concentrations, little or no formaldehyde is predicted to reach the lower respiratory tact. The efficient scrubbing of formaldehyde in the nose and upper respiratory tract means, however, that the severity of cytotoxic effects increases monotonically with increasing concentration.

CONCLUSION

A sensitive analysis of the expected human inhalation exposure dose response for formaldehyde-induced respiratory tract cytotoxicity is presented. The departure from a constant $C \times T$ relationship in studies with experimental animals and the inhibitory effect of formaldehyde on cell replication at low levels of exposure suggest that the results presented here can be used to derive standards for continuous exposure without the need for $C \times T$ adjustments. The sensitivity of the analysis derives from the use of a computer modeling approach and from consideration of the effect of working level on the expected human dose response. Interestingly, there is a general correspondence between the predicted dose response for cytotoxicity described in this report and results of human studies of sensory irritation (Bender, 2001). For both cases, the most sensitive responses are reported near 1 ppm with the severity of the response increasing fairly rapidly as concentrations increase above that level. This concordance increases confidence that exposure standards intended to protect against cytotoxic effects of formaldehyde will also protect against other acute formaldehyde-responsive endpoints.

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