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The bottom-up approach to bounding potential low-dose cancer risks from formaldehyde: An update

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ABSTRACT

In 2013, we proposed a novel bottom-up approach to bounding low-dose cancer risks that may result from small exogenous exposures to chemicals that are always present in the body as a result of normal biological processes. The approach utilizes the background cancer risk and the background (endogenous) concentration of a cancer-related exposure biomarker in specific target tissues. After allowing for statistical uncertainty in these two parameters, the ratio of the background risk to background exposure provides a conservative slope factor estimate that can be utilized to bound the added risk that may be associated with incremental exogenous exposures. Our original bottom-up estimates were markedly smaller than those obtained previously by the US Environmental Protection Agency (USEPA) with a conventional top-down approach to modeling nasopharyngeal cancer and leukemia mortality data from a US worker cohort. Herein we provide updated bottom-up estimates of risk for these two cancers that are smaller still, and rely upon more robust estimates of endogenous and exogenous formaldehyde-DNA adducts in monkeys and a more robust estimate of the DNA adduct elimination half-life in rats, both obtained very recently. We also re-examine the worker mortality data used by USEPA in developing its estimate of human leukemia incidence from lifetime exposure to 1 ppm airborne formaldehyde. Finally, we compare a new bottom-up slope estimate of the risk of rat nasal cancer with conventional top-down estimates obtained with empirical dose-response modeling of rat nasal cancer bioassay data.

1. Introduction

In 2013, we proposed a novel bottom-up approach to bounding the low-dose human cancer risks that may arise from small exogenous exposures to chemicals that are always present in the body as a result of normal biological processes such as metabolism (Starr and Swenberg, 2013). This approach utilizes two parameters: 1) the background cancer risk (P_0) in a specific target tissue, and 2) the background (endogenous) concentration (C_0) of a cancer-related exposure biomarker, such as a specific DNA adduct, measured in the same tissue. After adjusting appropriately for statistical uncertainty in these parameters by replacing the central, i.e., maximum likelihood, estimates of P_0 and C_0 with their corresponding upper (P_{0U}) and lower (C_{0L}) confidence bounds, the ratio P_{0U}/C_{0L} provides a conservative cancer risk slope factor estimate that can be utilized to bound the added risk that may be associated with an incremental steady-state exogenous exposure (C_{xss}). The approach assumes that this bound on added risk (AR_U) is predominantly linear near C_0 and given by the equation:

$$AR_U = (P_{0U}/C_{0L}) \cdot C_{xss}.$$

Because the bottom-up approach makes an implicit assumption that all of the background cancer risk P_0 is attributable to the background endogenous exposure C_0, the resulting bound on added risk is "worst case" in that regard.

Strengths of our bottom-up approach are that it 1) is consistent with the "additivity to background" concept; 2) yields upper-bound risk estimates that are linear, and 3) requires only information on background risk, background (endogenous) exposure, and the additional steady-state exogenous exposure in order to be implemented. The bottom-up approach thus provides an independent

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"reality check" on low-dose added risk estimates derived with the typical top-down approach of fitting dose-response models to high-dose human or laboratory animal cancer data. The key biotechnological advance that underpins this novel approach is the extraordinary ability to distinguish between and separately quantify target tissue exposures that arise from unlabeled internal (endogenous) sources and dual stable isotope-labeled external (exogenous) sources, even when the endogenous exposure is substantially larger and more uncertain than the steady-state exogenous exposure of interest (c.f., Lu et al., 2010, 2012; Moeller et al., 2011; Swenberg et al., 2011, 2013; Yu et al., 2015).

When we introduced the bottom-up approach (Starr and Swenberg, 2013), we illustrated its application with an analysis of potential human cancer risks posed by inhalation exposures to formaldehyde. We used recent US national mortality statistics for nasopharyngeal cancer, Hodgkin lymphoma, and leukemia for estimates of P_0 and P_{0U}. We used short-term measurements of specific formaldehyde-DNA adducts (N_2-hydroxymethyl-dG (N2-HOMe-dG) adducts) in the presumptive target tissues for these cancers (nasal respiratory epithelium, blood, and bone marrow, respectively) in monkeys (Moeller et al., 2011), a surrogate species for humans. We assumed that the measured endogenous adduct concentrations were at steady-state, since endogenous exposure is continuous and ongoing at all times. We then extrapolated from time-specific exogenous adduct concentrations (measured in monkeys after 2 ppm formaldehyde exposure for 6 h on two consecutive days) to the steady-state values (C_{xss}) that would be expected to arise from continuous 24/7 exposure using a simple one compartment pharmacokinetic model of DNA adduct formation and elimination. The 63 h elimination half-life that we utilized in the kinetic model had been derived previously from limited short term data obtained with rats (Swenberg et al., 2013).

Our original bottom-up estimates of added risk were markedly smaller than those that had been obtained by the US Environmental Protection Agency (USEPA, 2010) with a conventional top-down approach that utilized data from occupational cohort mortality studies of formaldehyde-exposed workers (Hauptmann et al., 2004; Beane-Freeman et al., 2009). For nasopharyngeal cancer mortality, our bottom-up bound on added risk from lifetime continuous exposure to 1 ppm formaldehyde (0.038×10^{-2}) was nearly 29-fold lower than USEPA's "plausible" upper bound estimate for nasopharyngeal cancer incidence (1.1×10^{-2}), while our bottom-up bound for the added risk of developing leukemia ($<3.9 \times 10^{-6}$) from the same lifetime exposure was more than 14,000-fold lower than the corresponding USEPA estimate for leukemia incidence (5.7×10^{-2}).

Herein we provide updated estimates of added risk for leukemia and nasopharyngeal cancer using the same bottom-up approach. The new estimates rely upon more robust estimates of tissue-specific endogenous and exogenous formaldehyde-DNA adducts in monkeys and a more robust estimate of the DNA adduct elimination half-life in rats, both of which were obtained very recently by Yu et al. (2015). We also examine more closely the data that were used by USEPA (2010) in developing its estimate of leukemia incidence from lifetime exposure to 1 ppm airborne formaldehyde.

Finally, we compare a newly derived bottom-up added risk estimate for rat nasal cancer incidence with a conventional top-down estimate that we obtained with empirical dose-response modeling of pooled nasal cavity squamous cell carcinoma data that were taken from two formaldehyde carcinogenicity bioassays (Kerns et al., 1983; Monticello et al., 1996) and selected historical control groups from National Toxicology Program (NTP) inhalation bioassays (Subramaniam et al., 2007). These data provide the very best currently available information, in terms of the range of airborne formaldehyde concentrations studied and the accuracy and precision of both exposure concentrations and tumor incidence, that can be used in evaluating the performance of the bottom-up and top-down extrapolation approaches to bounding potential cancer risks from low level airborne formaldehyde exposures.

2. Materials and methods

2.1. Human cancer risk estimates

For background lifetime risk of death from nasopharyngeal cancer, we employed the same risk estimate that we used previously in Starr and Swenberg (2013), namely, 7.25×10^{-4}, which was taken from USEPA's 2 June 2010 draft formaldehyde assessment (see Table C-1, p C-3 and Section 5.2.2 in USEPA (2010)). The 2010–2012 all races background lifetime risk of developing leukemia (ICD-10 codes C91-C95) absent exogenous formaldehyde exposure (1.47×10^{-2}) was taken from Table 13.20 of the *SEER Cancer Statistics Review 1975–2012* (Howlader et al., 2015). This value is about 13% higher than the previous SEER estimate (1.30×10^{-2}) for the period 2005–2007, which we employed previously in Starr and Swenberg (2013). Because these background lifetime risk estimates apply to the entire US population, their uncertainty is extremely small, i.e., P_0 and P_{0U} are practically identical, differing by a few percent at most, and we have therefore utilized P_0 in all of our bottom-up calculations of human risk.

Also, stratified leukemia (ICD-8 codes 204-207) mortality data were taken from the National Cancer Institute (NCI) occupational cohort mortality study of formaldehyde-exposed workers (Beane-Freeman et al., 2009). Observed and expected numbers of leukemia deaths in four cumulative formaldehyde exposure strata (unexposed, >0 – <1.5 ppm-yr, 1.5–5.5 ppm-yr, > 5.5 ppm-yr) were utilized herein to consider whether top-down dose-response modeling and extrapolation of predicted risks could be justified scientifically. These data and corresponding Standardized Mortality Ratios (SMRs) for the same strata were kindly provided by Dr. Kenneth A. Mundt, Ramboll ENVIRON International Corporation (personal communication). Dr. Mundt also provided us with stratum-specific mean cumulative exposure values for these strata that were utilized in top-down Poisson regression dose-response modeling of leukemia mortality in the NCI cohort (Beane-Freeman et al., 2009). All modeling calculations were implemented in Microsoft Excel 2007. Four polynomial dose-response models (constant, linear with unit intercept, linear, and quadratic) were fit by maximum likelihood to the stratified mortality data using the maximum likelihood method implemented with the Excel Solver optimization routine.

2.2. Rat nasal cancer risk estimates

The background lifetime risk estimate for nasal squamous cell carcinoma (SCC) in Fischer 344 rats (1/4949) was taken from Subramaniam et al. (2007), who combined nasal SCC incidence in the concurrent control groups from two formaldehyde inhalation bioassays (Kerns et al., 1983; Monticello et al., 1996) with that in several historical control groups from a series of National Toxicology Program (NTP) inhalation bioassays (see Table 1 in Subramaniam et al. (2007) and related discussion for details). We also utilized the SCC dose-response data from Kerns et al. (1983) and Monticello et al. (1996) as combined by Subramaniam et al. (2007) in our top-down dose-response modeling of tumor incidence versus estimated steady-state formaldehyde-DNA adduct concentrations in rat nasal respiratory epithelium. A Weibull model, modified to allow for the possibility of a linear term in addition to the model's standard power law dose-dependence, was fit to the rat tumor data up to and including that for the 10 ppm

dose group using the method of maximum likelihood and the Microsoft Excel 2007 Solver optimization routine, while a multistage model was fit to the same data using the Global 82 computer program (Howe and Crump, 1986).

2.3. Endogenous and exogenous formaldehyde-DNA adducts

Species- and tissue-specific data for endogenous formaldehyde-DNA adducts (N^2-HOMe-dG mono adducts) in air control monkeys (cynomolgus macaques) (n = 4 for nasal tissue; n = 10 for scraped bone marrow), and air control Fischer 344 rats (n = 8 for nasal tissue), were taken from Yu et al. (2015). The air control monkeys were whole body-exposed to filtered air for 6 h on 2 consecutive days. The air control rats received 28 consecutive days of 6 h per day nose-only exposure to filtered air.

Yu et al. (2015) collected monkey nasal tissue samples from 5 distinct areas of the nasal cavity (dorsal mucosa, nasapharynx, septum, anterior maxillary turbinate, and posterior maxillary turbinate). All possible (n = 10) paired sample t-tests were conducted to determine if there were any significant differences in endogenous adduct concentrations with nasal cavity location. No such differences were found, even without adjusting for the multiple comparisons, so the area-specific values were combined into a nasal cavity mean value for each monkey, and these means were then used to calculate a single overall grand mean value and standard error of estimate.

Tissue-specific concentrations of stable isotope-labeled exogenous formaldehyde-DNA adducts in the nasal tissues of exposed monkeys measured following 6 h exposures to 6 ppm formaldehyde on two consecutive days were also taken from Yu et al. (2015). However, these adducts were not detected in any monkey bone marrow sample (average sample size = 22.78 nMol dG (Dr. Rui Yu, personal communication)). We therefore utilized Yu et al.'s analytical method detection limit for these adducts on column (0.5 amol) to specify an upper bound on how many exogenous adducts could be present but still remain undetected in the average amount of dG present in scraped bone marrow samples. Yu et al.'s 0.5 amol detection limit on column thus corresponds to 0.5×10^{-18}/ 22.78×10^{-9}, or 0.000219 adducts per 10^7 dG.

Finally, data for dual stable isotope-labeled exogenous formaldehyde-DNA adducts in rat nasal tissue following single 6 h exposures to airborne formaldehyde concentrations 0.7, 2.0, 5.8, or 9.1 ppm were taken from Table 1 of Lu et al. (2010) for use in top-down dose-response modeling of the rat nasal tumor incidence data. The Lu et al. (2010) values for 0.7 and 2 ppm formaldehyde were used directly. The Lu et al. (2010) values for 5.8 and 9.1 ppm formaldehyde were extrapolated upward linearly to the closest bioassay formaldehyde concentrations reported in Table 1 of Subramaniam et al. (2007), namely, 6.01 and 9.93 ppm.

3. Results

3.1. Bottom-up nasopharyngeal cancer and leukemia mortality risk estimates

Table 1 summarizes the elements involved in using the bottom-up approach to predict potential human upper bound risks of death from nasopharyngeal cancer and the development of leukemia that might arise from continuous lifetime exposure to 1 ppm formaldehyde. Both of these bottom-up risk estimates are reduced (by about 1.4-fold for nasopharyngeal cancer and 3.1-fold for leukemia) relative to the estimates presented previously in Starr and Swenberg (2013). The 1.4-fold reduction in nasopharyngeal cancer mortality risk is due primarily to a 1.7-fold increase in the estimate of C_{0L} in monkey nasal tissues, which decreases the bottom-up slope estimate P_0/C_{0L}. This increase in the estimate of C_{0L} is due at least in part to the fact that the endogenous adduct concentration C_0 in monkey tissue is more tightly estimated with the new data from Yu et al. (2015).

The 3.1-fold reduction in the bottom-up bound on leukemia risk is due primarily to the increased sensitivity of the analytical method employed by Yu et al. (2015), who now have an on-column detection limit of 0.5 attoMol, 40-fold lower than the previous value (20 attoMol) reported by Moeller et al. (2011). This reduction is counterbalanced in part by a decrease in the quantity of DNA collected per sample, and an approximately 60% decrease in the estimates of C_0 and C_{0L} in scraped bone marrow relative the previous estimates reported by Moeller et al. (2011).

Use of the new DNA adduct data from Yu et al. (2015) enhanced the contrast between the bottom-up risk estimates and USEPA's top-down estimates. The bottom-up estimate of nasopharyngeal cancer mortality risk from lifetime exposure to 1 ppm formaldehyde is now over 40-fold lower than the USEPA (2010) estimate of 1.1%, while the corresponding bottom-up estimate of developing leukemia is now more than 45,000-fold lower than the USEPA (2010) estimate of 5.7%. These profound contrasts call the scientific credibility of the Agency's estimates into serious question, especially for the lifetime risk of developing leukemia.

3.2. Top-down dose-response analyses of human leukemia mortality

Table 2 and Fig. 1 provide the data utilized in our top-down dose-response modeling of human leukemia mortality. It is readily apparent that 1) the 50% deficit in leukemia mortality in the unexposed stratum is nearly statistically significant (95% confidence interval = (0.24, 1.04), and 2) there is little, if any, evidence of a positive dose-response relationship between leukemia mortality and cumulative formaldehyde exposure amongst exposed person-years (SMR = 0.96, 0.96, 1.27 for the three strata with nonzero cumulative exposure).

Table 3 and Fig. 1 summarize results from our Poisson regression modeling efforts. The χ^2 Goodness of Fit test p-values, all greater than p = 0.05, indicate that each of the four polynomial models provided adequate fits to the data. However, the unconstrained linear model (Fig. 1b), failed to provide a significantly better fit to the data than did the pure intercept model (Fig. 1a) (χ^2 likelihood ratio test p-value = 0.089, 1 degree of freedom (df)), and the quadratic model (Fig. 1c) failed to provide a significantly better fit to the data than did the linear model (χ^2 likelihood ratio test p-value = 0.760, 1 df) or the pure intercept model (χ^2 likelihood ratio test p-value = 0.255, 2 df). In other words, the pure intercept model, a simple horizontal (zero slope) straight line, provided an entirely satisfactory description of the data, consistent with the lack of evidence for a positive association between leukemia mortality and cumulative formaldehyde exposure in this cohort. These results are in complete agreement with the nonsignificant log-linear trend test statistics reported previously by Beane-Freeman et al. (2009) for their unstratified analysis of the NCI leukemia mortality data, namely, p-value = 0.08 using all person-years, and p-value = 0.12 using only exposed person-years.

In summary, our stratified dose-response analyses confirm the previous findings of Beane-Freeman et al. (2009) that there is no substantive evidence of a positive dose-response relationship between leukemia mortality and cumulative formaldehyde exposure in the NCI cohort. In such circumstances, top-down extrapolations from the leukemia mortality observed in this cohort to potential risks arising from far lower levels of chronic environmental formaldehyde exposures are simply not scientifically justified.

Table 1
Estimated lifetime risks of death from nasopharyngeal cancer and development of leukemia from continuous lifetime inhalation exposure to 1 ppm formaldehyde, as re-estimated with the bottom-up approach using more robust input data and, alternatively, by USEPA using top-down linear extrapolation from epidemiologic data (as taken from Table 6-3, pp 6.41 – 6.42, of the Agency's draft toxicological assessment (USEPA, 2010)). For comparison, previous bottom-up estimates of slope, steady-state exogenous formaldehyde-DNA adduct concentrations, and risk from Starr and Swenberg (2013) are shown in parentheses. All formaldehyde-dG adduct concentrations are expressed as the number of adducts per 10^7 dG in cynomolgus monkey nasal tissues or scraped bone marrow.

Endpoint	Background Risk P_0	$C_0 \pm seC_{0L}$	B-U slope[a] P_0/C_{0L}	C_{x30} @ 6 ppm C_{xss} @ 6 ppm	C_{xss} @ 1 ppm	Bottom-up[a] AR_U @ 1 ppm	EPA UCL_{95} Risk @ 1 ppm
Nasopharyngeal Cancer	7.25×10^{-4}	3.84 ± 0.18	(3.44×10^{-4})	0.363 ± 0.015	(1.10)	(3.80×10^{-4})	1.1×10^{-2}
		3.55	2.04×10^{-4}	7.935 ± 0.328	1.32	2.69×10^{-4}	
Leukemia	1.47×10^{-2}	10.18 ± 0.43	(0.85×10^{-3})	$<2.19 \times 10^{-4}$	$(<4.56 \times 10^{-3})$	$(<3.90 \times 10^{-6})$	5.7×10^{-2}
		9.48	1.55×10^{-3}	$<4.79 \times 10^{-3}$	$<7.98 \times 10^{-4}$	$<1.24 \times 10^{-6}$	

[a] For human background risks, the central (P_0) and upper bound (P_{0U}) estimates are nearly identical due to the very large sample size (the entire US population), so bottom-up slope and added risk estimates utilize P_0, not P_{0U}.

Table 2
Observed and expected deaths and SMRs for stratified leukemia mortality data (ICD codes 204-207) versus stratum-specific mean cumulative exposure in the NCI cohort with followup through 2004, as constructed by Dr. Kenneth Mundt, Ramboll ENVIRON Corporation (personal communication).

Exposure stratum	Exposure, ppm-years[a]	Observed deaths	Expected deaths[b]	SMR[b]	95% confidence interval
0	0	7	14.1	0.50	(0.24, 1.04)
>0 to <1.5	0.342	63	65.5	0.96	(0.75, 1.23)
1.5 to < 5.5	2.963	23	23.9	0.96	(0.64, 1.45)
>5.5	16.656	30	23.7	1.27	(0.89, 1.81)

[a] Mean cumulative exposures within each stratum were calculated using a 2-year lag.
[b] Expected deaths and the SMR were calculated using SEER CanQues US mortality rates for 1970–2009.

3.3. Bottom-up rat nasal cancer risk estimates

Based on the cumulative binomial distribution, the estimated background incidence of rat nasal cancer taken from Subramaniam et al. (2007), namely, $P_0 = 1/4949 = 2.021 \times 10^{-4}$, has an upper 95% confidence bound (P_{0U}) of 9.582×10^{-4}, i.e., nearly 5-fold higher than P_0. Furthermore, the number of endogenous N^2-HOMe-dG adducts in rat nasal tissue taken from Yu et al. (2015), namely, $C_0 = 2.84$ per 10^7 dG, has a lower 95% confidence bound of $C_{0L} = 2.84 - 1.645 \times 0.54/\sqrt{8} = 2.53$ per 10^7 dG. Thus, the bottom-up bound on the added risk slope factor for rat nasal cancer is given by $P_{0U}/C_{0L} = 3.793 \times 10^{-4}$ per 10^7 dG. Table 4 presents the bottom-up added risk estimates (AR_U) for lifetime exposure of rats using the standard chronic inhalation bioassay exposure regimen (6 h/day, 5 days/week) to selected airborne formaldehyde concentrations up to 2 ppm.

3.4. Top-down dose-response analyses of rat nasal cancer data

Table 5 summarizes the dose-response data utilized in our top-down modeling of rat nasal cancer. Column 1 provides the airborne formaldehyde concentrations reported in Table 1 of Subramaniam et al. (2007). Column 2 shows the number of exogenous N^2-HOMe-dG adducts present in rat nasal epithelium following a single 6 h exposure to each of those airborne formaldehyde concentrations (C_{x6}) as reported by Lu et al. (2011) for 0., 0.7, and 2.0 ppm, or as extrapolated linearly upward to values for 6.01 and 9.93 ppm formaldehyde from the values Lu et al. (2011) reported for 5.8 and 9.1 ppm.

The exogenous formaldehyde-DNA adduct concentrations following 6 h of exposure were converted to the steady-state concentrations C_{xss} (shown in Column 3 of Table 5) that would arise from chronic 6 h/day 5 day/week exposure using the 171 h adduct elimination half-life ($T_{1/2}$) reported by Yu et al. (2015) and their one compartment pharmacokinetic model, which assumes that the rate of adduct formation and clearance is constant throughout life. This model implies the following mathematical relationship between C_{xss} and C_{x6}:

$$C_{xss} = C_{x6}/(1 - \exp(-6/T)) \times (6/24) \times (5/7),$$

where $T = T_{1/2}/\ln(2) = 246.7$ h, and where the multiplicative factors of (6/24) and (5/7) are included to account for the discontinuous 6 h/day 5 day/week bioassay exposure regimen. The total N^2-HOMe-dG steady-state adduct burden shown in column 4 of Table 5 was obtained by summing these adjusted C_{xss} values and the endogenous background adduct concentration C_0 (2.84 per 10^7 dG), also taken from Yu et al. (2015).

Dose-response modeling results are presented in Table 6. Goodness of fit statistics for the modified Weibull and multistage models were virtually identical ($\chi^2(2) = 0.108$ and 0.107, respectively), and both model fits were excellent ($p = 0.947$ and 0.948, respectively) and essentially indistinguishable from one another. The maximum log-likelihood values for the two models (−80.2370 and −80.2375, respectively) were also virtually identical.

Both of the fitted models had a substantial intercept parameter (a_0), indicating that only a small fraction of the background cancer risk (approximately 10%) was attributed by the fitting process to the endogenous rat nasal tissue adduct burden C_0., with the balance of the background risk being attributed to sources other than the background adduct burden. In addition, the linear terms in both models were driven to zero during the model optimization process. In fact, apart from the intercept parameter a_0, the only non-zero dose coefficients in the multistage model were for the fifth (a_5) and sixth (a_6) powers of dose, and the value of the power parameter p for the modified Weibull model was equal to 5.86. These results reflect the highly nonlinear dose-dependence of the rat nasal tumor response. They are also entirely consistent with the top-down dose-response modeling results we obtained with airborne formaldehyde concentration as the dose-metric (data not shown).

The last two rows of Table 6 present maximum likelihood estimates of risk and the slope of the top-down dose-response models at the three rat nasal tissue adduct concentrations (2.84, 3.39, and 4.25 adducts per 10^7 dG), corresponding to 0, 1, and 2 ppm airborne formaldehyde, respectively. It is noteworthy that both of the fitted models predict risks at 2.84 (0 ppm) and 3.39 adducts per 10^7 dG (1 ppm) that are actually smaller than the observed background

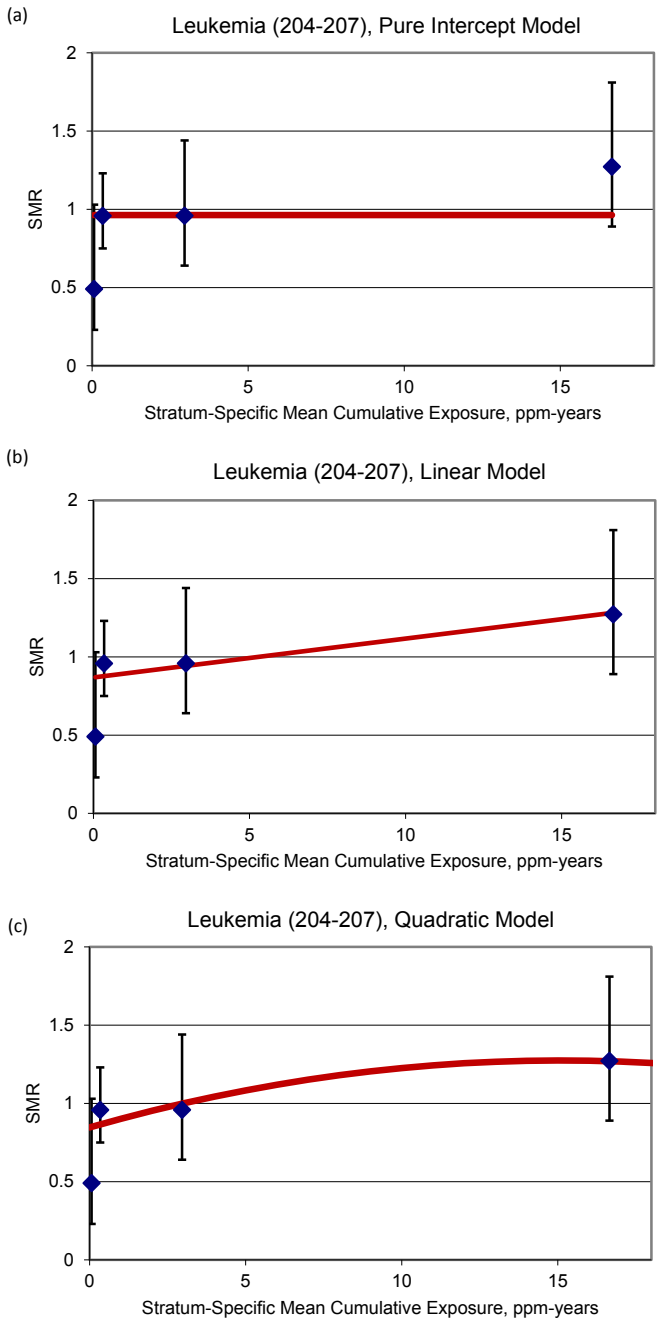

Fig. 1. 1a-1c. Plots of Standardized Mortality Ratios for leukemia in the NCI cohort stratified on cumulative formaldehyde exposure. Also shown are the best fitting pure intercept (1a), linear (1b), and quadratic (1c) dose-response models as estimated with Poisson regression (see Tables 2 and 3 for additional information).

Table 3
Poisson regression model fits to the stratified human leukemia mortality data summarized in Table 1.

Model	Parameters	GOF[a] χ^2/df	GOF p-value	ln likelihood
b_0	b_0 0.963	5.658/3	0.129	322.699
$1 + b_1 X$	b_1 0.0150	4.054/3	0.256	323.982
$b_0 + b_1 X$	b_0 0.869 b_1 0.0248	2.859/2	0.239	324.145
$b_0 + b_1 X + b_2 X^2$	b_0 0.847 b_1 0.0567 b_2 −0.00188	2.831/1	0.092	324.192

[a] GOF: Goodness of Fit.

risk of 2.02×10^{-4}. The fitted models only predict risks that begin to exceed the observed background risk at or above 3.9 adducts per 10^7 dG, which is only 10% lower than the 4.25 adducts per 10^7 dG that result from chronic exposure to 2 ppm formaldehyde.

All of the slopes of the top-down models shown in Table 6 are smaller than our bottom-up slope central slope of estimate (P_0/C_0), namely, (1/4949)/2.84 per 10^7 dG, or 7.11482×10^{-5} per 10^7 dG. Furthermore, the top-down model slopes are substantially smaller than our bottom-up upper bound slope (P_{0U}/C_{0L}), namely 3.79×10^{-4} per 10^7 dG, even at a total adduct burden of 4.25 per 10^7 dG (2 ppm). Ratios of our bottom-up upper bound slope to the maximum likelihood slope estimate from the modified Weibull (multistage) model are 57.0 (43.6) at 2.84×10^7 per dG (0 ppm), 24.2 (19.6) at 3.39 per 10^7 dG (1 ppm), and 8.1 (7.0) at 4.25 per 10^7 dG (2 ppm), respectively. Our bottom-up upper bound estimate of the low-dose slope of the rat nasal cancer response thus exceeds the top-down model slope estimates by nearly an order of magnitude or more up to 2 ppm formaldehyde.

4. Discussion

4.1. Bottom-up human cancer risk estimates

Our updated bottom-up upper bound risk estimates for human nasopharyngeal cancer and leukemia mortality rely on more robust data for key input parameters, namely, extensive additional measurements of endogenous and exogenous N_2-hydroxymethyl-dG adduct numbers in the relevant target tissues of a human surrogate species, cynomolgus monkeys, as well as a significantly improved estimate of the half-life of these adducts obtained from male rat nasal tissue data collected following repeated 6 h exposures to 2 ppm formaldehyde for up to 28 consecutive days (Yu et al., 2015). However, at present, an assumption of cross-species equivalence of 1) tissue-specific formaldehyde-DNA adduct numbers, and 2) the elimination half-life for these adducts is necessary if the available nonhuman data are to be utilized in quantitative risk assessments of human exposures to formaldehyde. This cross-species equivalence assumption still needs to be validated and/or replaced with measurements of formaldehyde-DNA adducts in human tissues, and we are optimistic that such data will be obtained in the near future.

In addition, it is important to note that use of the short-term DNA adduct data to construct a dose metric suitable for lifetime cancer risk extrapolation necessitates the assumption that formaldehyde-DNA adduct formation and clearance, be it from either endogenous or exogenous formaldehyde sources, occurs throughout life at the same steady-state rate that we have inferred from the short-term data via pharmacokinetic modeling. We believe this is a reasonable assumption, but it has not yet been validated and/or replaced with longer term measurements in human, subhuman primate, or rodent tissues. Fuller discussions of the strengths, limitations, and relevance of short-term DNA adduct data to cancer risk assessment can be found in Jarabek et al. (2009), Himmelstein et al. (2009), Swenberg et al. (2011, 2013), and Pottenger et al. (2014).

While our updated bottom-up risk estimates have served to heighten the marked contrasts between our previous estimates and the corresponding USEPA (2010) risk estimates, the changes from our previous estimates, namely, a 1.4-fold reduction in nasopharyngeal cancer mortality, and 3.15-fold reduction in leukemia mortality, are not dramatic, with the larger difference for leukemia mortality being due primarily to the markedly lower detection limit for the analytical method that is now being used to quantify formaldehyde-DNA adduct numbers.

Is USEPA's leukemia upper bound slope factor estimate of 5.7%

Table 4
Bottom-up estimates of added risk of nasal cancer to rats exposed chronically to selected airborne formaldehyde concentrations for 6 h/day, 5 days/week.

Airborne concentration, ppm	C_{x6}, adducts per 10^7 dG	C_{xss}, adducts per 10^7 dG	AR_U, bottom-up Bound on added risk[a]
0	0	0	0
0.1	0.006	0.04	1.57×10^{-5}
0.7	0.039	0.29	1.10×10^{-4}
1.0	0.095	0.71	2.67×10^{-4}
2.0	0.190	1.41	5.35×10^{-4}

[a] $AR_U = (P_{0U}/C_{0L}) \times C_{xss}$.

Table 5
Data for rat nasal tissue formaldehyde-DNA adducts and squamous cell carcinoma incidence that were utilized in top-down dose-response modeling.

Air concentration, ppm	C_{x6},[a] adducts per 10^7 dG	C_{xss},[b] adducts per 10^7 dG	C_{Tot},[c] adducts per 10^7 dG	Squamous cell carcinoma incidence[d]
0	0	0	2.84	1/494
0.7	0.039	0.29	3.13	0/107
2.0	0.19	1.41	4.25	0/353
6.01	1.08	8.03	10.87	3/343
9.93	2.22	16.50	19.34	22/103

[a] Taken from Lu et al. (2011). The C_{x6} values for 6.01 and 9.33 ppm were linearly extrapolated upward from the values for 5.8 and 9.1 ppm reported in Table 1 of Lu et al. (2011).
[b] Quasi-steadystate values $C_{xss} = C_{x6}/(1\text{-}\exp(\text{-}6/T)) \times 6/24 \times 5/7$, where $T = T_{1/2}/\ln(2)$, and $T_{1/2} = 171$ h.
[c] $C_{Tot} = C_{xss} + C_0$.
[d] Pooled rat nasal tumor incidence taken from Subramaniam et al. (2007).

Table 6
Parameter estimates and fitting statistics for modified Weibull and Multistage models fit by maximum likelihood (ML) to the rat nasal tumor data provided in Table 5. Also shown are model-predicted risks and slopes for total formaldehyde-DNA adduct burdens that result from chronic 6 h/day 5 day/week exposure to exogenous formaldehyde concentrations of 0, 1, and 2 ppm. All formaldehyde-DNA adduct concentrations are expressed as the number of adducts per 10^7 dG in rat nasal respiratory epithelium.

Modified Weibull Model		Multistage Model	
a_0	0.182190×10^{-3}	a_0	0.180526×10^{-3}
a_1	0	a_1, a_2, a_3, a_4, a_7	0
a_p	0.721100×10^{-8}	a_5	0.134914×10^{-7}
P	5.84854	a_6	0.389691×10^{-8}
$\chi^2(2)$	0.108148		0.107024
GOF p-value	0.947362		0.947895
Max Likelihood	−80.2370		−80.2375
ML Risk			
@ 2.84 (0 ppm)	1.84973×10^{-4}		1.85046×10^{-4}
@ 3.39 (1 ppm)	1.91269×10^{-4}		1.92462×10^{-4}
@ 4.25 (2 ppm)	2.14807×10^{-4}		2.22173×10^{-4}
ML Slope			
@ 2.84 (0 ppm)	6.65112×10^{-6}		8.70653×10^{-6}
@ 3.39 (1 ppm)	1.56911×10^{-5}		1.93734×10^{-5}
@ 4.25 (2 ppm)	4.69589×10^{-5}		5.44163×10^{-5}

per ppm formaldehyde scientifically defensible? We think not. This estimate was constructed using the NCI cohort data described by Beane-Freeman et al. (2009), but that study reported no statistically significant association between leukemia mortality and workers' cumulative formaldehyde exposure, with non-significant trend tests of mortality versus cumulative exposure using all person-years ($p = 0.08$) or only exposed person-years ($p = 0.12$). Our Poisson regression analyses of stratified data from the NCI cohort (see Table 3 and Fig. 1) confirm the absence of any significant trend in leukemia mortality with cumulative exposure. Indeed, as noted earlier, linear and quadratic models of leukemia mortality versus cumulative exposure failed to improve significantly upon the fit of a pure intercept model that implies no relationship whatsoever between leukemia risk and cumulative formaldehyde exposure. To us, it seems therefore to be altogether inappropriate to utilize top-down dose-response modeling, as USEPA (2010) has done, to estimate low-dose leukemia risks as a function of cumulative formaldehyde exposure when there is no substantive evidence for a positive dose-response relationship between leukemia risk and cumulative formaldehyde exposure. Simply put, there is no dose-response to be modeled.

Furthermore, consideration needs to be given to the fact that whole-body exposure of monkeys to 6 ppm airborne formaldehyde for 6 h on two consecutive days failed to produce detectable exogenous formaldehyde-DNA adducts in bone marrow. This is compelling evidence that little, if any, inhaled exogenous formaldehyde reaches the bone marrow. There is thus no reason to expect an increase in leukemia mortality from exogenous formaldehyde exposure, even in the highest cumulative exposure stratum (>5.5 ppm-years) of the NCI cohort, for which the average cumulative exposure was 16.656 ppm-years (see Table 2). This value corresponds to an average lifetime continuous airborne formaldehyde concentration of approximately 0.1 ppm (assuming a workday inhalation volume of 10 m^3 out of a full 24 h inhalation volume of 20 m^3) and an 80 year lifespan). This implies an upper limit on the corresponding steady-state exogenous N^2-hydroxymethyl-dG adduct concentration C_{xss} of at most 7.98×10^{-5} per 10^7 dG in bone marrow (7.98×10^{-4} per 10^7 dG per ppm $\times$ 0.1 ppm). Thus, the steady-state exogenous adduct burden in bone marrow could only be about 0.78×10^{-5} of the endogenous adduct burden (7.98×10^{-5} per 10^7 dG/10.18 per 10^7 dG). It is difficult to imagine how an increase in the adduct burden from C_0 to 1.0000078 C_0, could cause a greater than doubling of the SMR for leukemia, from 0.50 in unexposed workers to 1.27 in the most highly exposed workers (see SMR leukemia data provided in Table 2). Such a result is simply not credible.

4.2. Regulatory agency concerns with the bottom-up approach

Since our initial publication describing the bottom-up approach to human health risk assessment (Starr and Swenberg, 2013), there have been numerous opportunities to present and discuss the approach with regulators and the scientific community. We have met privately with USEPA staff on three separate occasions to describe the approach in detail and discuss the Agency's concerns

about it. In collaboration with Dr. Robinan Gentry, we made a case study presentation that included an illustration of how the bottom-up approach could be applied to formaldehyde at an Alliance for Risk Assessment workshop (Gentry et al., 2013). In addition, one of us (TBS) and Dr. Kenny S. Crump made invited point-counterpoint presentations on the bottom-up approach at a 2014 USEPA-sponsored "State-of-the-Science Workshop to Discuss Issues Relevant for Assessing the Health Hazards of Formaldehyde Inhalation". Finally, the substance of these latter two presentations was reprised in an exchange of letters to the editor of *Regulatory Toxicology and Pharmacology* (Crump et al., 2014; Starr and Swenberg, 2014).

In essence, USEPA and Crump et al. (2014) have rejected the bottom-up approach because it is linear, claiming that it is "highly plausible" for the dose-response relationship between cancer risk and the steady state target tissue DNA adduct concentration to be highly sublinear *even below* the endogenous level C_0, and claiming further that such sublinearity forces the slope of the dose-response relationship to exceed our bottom-up estimate (P_{0U}/C_{0L}) at all total DNA adduct concentrations equal to or greater than C_0.

These claims have no merit. First, there are no data regarding cancer risks below C_0, so *any* statements regarding the shape of the dose-response relationship below C_0 must be purely speculative. Indeed, the dose-response relationship below C_0 cannot even be investigated, because the endogenous DNA adducts that comprise C_0 are always present, even when there is no *exogenous* exposure. Furthermore, our bottom-up approach to bounding the cancer risk at low doses includes no assumptions whatsoever regarding the shape of the dose-response curve below C_0. Instead, it conservatively assumes only that 1) the upper bound on added risk at and slightly above C_0 is approximately linear, and that 2) all of the background risk P_0 might plausibly be attributable to the endogenous background exposure C_0.

As we noted in our response to Crump et al. (2014), one can always posit an alternative hypothetical dose-response relationship that exceeds *any* given empirically-derived upper confidence bound on extrapolated risk, including those that result from the standard top-down approach to cancer risk assessment that has been the common practice for many years (Starr and Swenberg, 2014). This should not be surprising because the values and coverage properties of confidence bounds on extrapolated risk depend critically on the assumed dose-response relationship from which these bounds are derived. If the assumed relationship is changed, either qualitatively (different model type) or quantitatively (different parameter values), then the confidence bounds will change with it, and there are few "theoretical" constraints on the shape of potential dose-response relationships save positivity, continuity, differentiability and a finite (possibly zero) slope at zero dose. Other regulatory agency constraints such as monotonicity (precluding hormesis), low-dose linearity, and/or restriction of the possibilities to a small number of "acceptable" empirical dose-response relationships, e.g., the Weibull and multistage dose-response models, may also be imposed.

What is surprising to us is that the USEPA and Crump et al. (2014), who have been long-time proponents of low-dose linearity as a key constraint on the shape of the dose-response relationship, have chosen instead to now embrace the completely antithetical position of predominant low-dose nonlinearity without any supporting evidence. Therefore, the critical question is this: what can be meaningfully inferred about potential cancer risks at and above the endogenous background exposure level C_0 from the limited tumor data that are available? To answer this question, we chose the best available formaldehyde carcinogenicity data set, namely, the pooled nasal tumor data for rats exposed chronically to airborne formaldehyde in two carcinogenicity bioassays.

4.3. Bottom-up and top-down risk estimates for rat nasal cancer

When we incorporated endogenous and exogenous formaldehyde adduct data into the standard top-down risk assessment approach and computed maximum likelihood estimates of low-dose nasal cancer risk in chronically exposed rats using a modified Weibull or the multistage model, we obtained results very different from those shown in Fig. 1 of Crump et al. (2014), where the hypothetical "true" sublinear dose-response relationship passes directly through the background risk (P_0) with a slope at C_0 and all higher exposure levels that appears to be much greater than our bottom-up bound on the slope, the ratio P_{0U}/C_{0L}.

As shown in our Table 6 and Fig. 2, both models (green curve) are nonlinear and concave upward even when they are extrapolated below C_0, but they are nevertheless extremely flat not only below C_0 but also for exposures that are substantially greater than C_0. Furthermore, the Weibull and multistage models both *underpredict* the observed nasal cancer risk at C_0, with most of the observed background risk, approximately 90% of it, being attributed by the models to sources *other* than the endogenous N^2-hydroxymethyl-dG adducts that are always present in rat nasal tissues. This is reflected clearly in the substantial intercept parameters (a_0) of both models as shown in Table 6.

Fig. 2 and Table 6 also show clearly that our bottom-up bound on risk (red line) exceeds, by a substantial margin, the fitted Weibull and multistage model risk estimates (green curve) up to and well beyond the 4.25 total formaldehyde-dG adducts per 10^7 dG that result from chronic 6 h/day 5 day/week inhalation exposure to 2 ppm formaldehyde. Fig. 2 and the results presented in Table 6 also show that the bottom-up bound on the slope of nasal cancer risk (slope of the red line in Fig. 2) is greater, by more than a 57-fold (43-fold) factor, than the slope of the Weibull (multistage) model at C_0. In addition, the bottom-up bound on the slope of predicted risk continues to exceed by substantial margins the slopes of the Weibull and multistage models at total formaldehyde-DNA adduct burdens up to and including 4.25 per 10^7 dG (2 ppm). The rat nasal tumor data, our bottom-up bounds on predicted risk and its slope, and the predicted risks from the fitted top-down dose-response models thus contradict completely the groundless speculations of Crump et al. (2014).

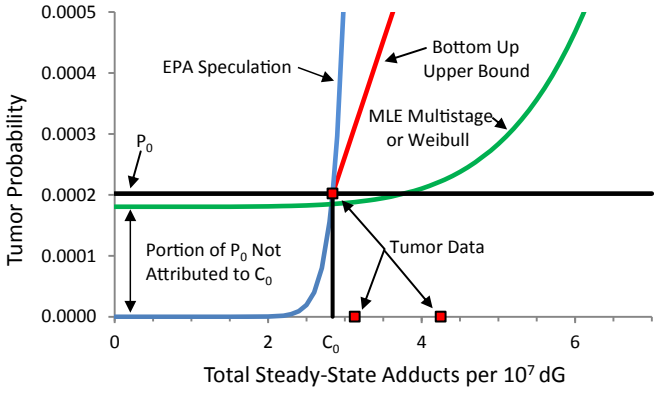

Fig. 2. Plots of best-fitting multistage and Weibull dose-response models (green curve) for rat nasal cancer. Also shown are the bottom-up upper bound on added risk (red line) and a depiction of the USEPA and Crump et al. (2014) speculative hypothesis (blue curve) that the "true" dose-response curve is highly nonlinear below, at, and above the endogenous background level of exposure (C_0). Nasal tumor incidence data for 0, 0.7, and 2.0 ppm formaldehyde are indicated by red squares. Observed background risk denoted by P_0.

4.4. Concluding remarks

We recognized from the outset that our bottom-up approach to bounding low-dose human cancer risks would likely not apply at exogenous exposures sufficiently high to induce nonlinear processes that amplify the carcinogenic response, such as saturation of metabolic pathways, cytotoxicity and tissue damage, and accelerated regenerative cell proliferation. With formaldehyde, such processes have been demonstrated to occur only at airborne concentrations greater than 2 ppm (Monticello et al., 1996). We therefore expect the bottom-up approach to provide valid bounding estimates of added risk from exposure to all airborne formaldehyde concentrations up to and including 2 ppm. The bottom-up approach may not always prove useful, but for cases where there is a substantial endogenous exposure in potential target tissues and little or no empirical evidence of a positive dose-response at low exogenous exposure levels, we are confident that this approach provides a robust and useful "reality check" on the typical top-down risk extrapolations from high-dose tumor data that are employed routinely in quantitative risk assessments.

Conflict of interest statement

TBS has served as a consultant on risk assessment issues related to formaldehyde for the American Chemistry Council. The formaldehyde research conducted by JAS has been funded in part by the NIEHS, the American Chemistry Council, Formacare, and the Texas Commission for Environmental Quality. The sponsors do not have access to research results until they have been accepted for publication. JAS has also served as a formaldehyde consultant to ENVIRON International.

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