Case Study

Endogenous Chemical Risk Assessment: Formaldehyde as a Case Example

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ABSTRACT

Conducting a dose-response assessment for endogenous compounds presents several challenges. The Science and Decisions (2009) report has indicated that it is possible that the dose-response curves for these types of compounds may be threshold-like, depending upon the magnitude of the background concentrations and toxic response. In addition, the dose-response curves may also appear to be linear if a detectable background level of toxicity occurs even without exogenous exposure and the exogenous exposure adds to or augments the background toxicity process, assuming the exogenous exposure does not induce an adaptive response. Formaldehyde provides an example of research and modeling activities being conducted to understand the endogenous concentrations of formaldehyde and the potential contribution of exogenous formaldehyde to the potential for health effects following inhalation exposure. The approaches demonstrate both the challenges in collecting the information needed to characterize internal doses in the low-concentration range, which is of significance to ambient exposure, as well as interpreting the results and the impact on understanding the dose-response for an endogenously present compound. These approaches can be extended to other compounds with endogenous DNA adducts that are identical to those produced by such chemicals as acetaldehyde, ethylene oxide and vinyl chloride. They may also be indicative of general phenomena related to endogenous DNA damage, as our DNA contains large amounts of endogenous DNA damage that are the reason for the well-known non-zero background of mutations, the biomarkers of effect that may be considered causal key events in carcinogenesis.

INTRODUCTION

In the Science and Decisions (NRC 2009) report, the National Research Council committee discussed consideration of the effects of exposures that add to background processes or endogenous concentrations when attempting to characterize the shape of the dose-response curve in the low-dose region (e.g., linear versus nonlinear). It is possible that the dose-response curves may be threshold-like, depending upon the magnitude of the background concentrations and toxic response. The dose-response curves may also appear to be linear if a detectable background level of toxicity occurs even without exogenous exposure and the exogenous exposure adds to or augments the background toxicity process. This assumes that the exogenous exposure will not induce an adaptive response.

New analytical methods are providing very sensitive and highly accurate quantitative data that allow quantitative assessment of these issues that were not readily available in the past. This includes the use of stable isotope exposures, measurement of DNA damage from common pathways such as oxidative stress and inflammation. Furthermore, mutation data for low exposures are finally being addressed, rather than the previous Hazard Identification studies that utilized high doses. What was previously plagued by uncertainty will be more readily addressed through appropriate dose-response modeling procedures.

Conducting a risk assessment for a compound that is endogenously present presents several challenges. First, methods are needed to quantify endogenous production and differentiate DNA damage arising from endogenous production of identical damage arising from exogenous exposure. Once such methods are developed and results are obtained, the additional challenge to the risk assessor is determining how to interpret the results and incorporate those results into an appropriate dose-response assessment. The risk assessor must also try to determine if the exogenous exposure can increase the endogenous levels sufficiently enough to create biological perturbations that culminate in detectable adverse effects.

Formaldehyde is present endogenously in all living cells; it is an essential metabolic intermediate. It also has numerous exogenous sources including vehicle emissions, building materials, and tobacco smoke, as well as through metabolism of

foods, chemicals and drugs. Recent hazard assessments for formaldehyde conducted by authoritative bodies (USEPA 2010; NTP 2011; IARC 2010) have identified concerns related to the potential for formaldehyde exposure to cause nasopharygeal cancers or lymphohematopoietic cancers and/or leukemias. The National Academy of Sciences (NAS) in reviewing recent assessments has noted:

> *"…formaldehyde is an endogenous compound and that this finding complicates assessments of the risk posed by inhalation of formaldehyde. This committee emphasizes that the natural presence of various concentrations of formaldehyde in target tissues remains an important uncertainty with regard to assessment of the additional dose received by inhalation."*

In the case of formaldehyde, there are several questions that need to be addressed in conducting a dose-response assessment:

- How can we accurately assess the risk of exogenous formaldehyde in the presence of a substantial background of endogenous formaldehyde?

- What is needed to conduct a dose-response assessment considering the "background" concentrations that are always present in biological systems?

- If a specific marker is used to differentiate endogenous from exogenous exposure, can this be a biomarker of exposure or a biomarker of effect (related to the mode of action)?

The current case study has multiple purposes, the first of which focuses on recent research results and ongoing dose-response and modeling analyses for formaldehyde as an example of methods for quantifying endogenous production and how the results can be incorporated into dose-response modeling and the evaluation of target tissue dosimetry. The research on formaldehyde includes recent work by Swenberg and colleagues (Lu et al. 2011, 2012, Moeller et al. 2011) that provides accurate characterization of endogenous versus exogenous DNA adducts following inhalation exposure to formaldehyde in rats and nonhuman primates. Initial efforts to incorporate this research into dose response assessment include:

- A "Bottom up" approach (Starr and Swenberg 2013) that provides an alternative to the "standard" top-down risk extrapolation from high dose animal or human cancer data.
- Incorporation of endogenous production of formaldehyde into the Biologically-Based Dose-Response Model (BBDR) reported by Conolly et al. (2003, 2004). These models currently do not consider endogenous production; however, recent

research has been conducted towards this incorporation and the initial findings (Schroeter et al. 2013) demonstrate the significant impact endogenous concentrations may have when characterizing the dose-response curve in the low concentration region.

Characterizing endogenous production of formaldehyde and consideration of these concentrations in conducting dose-response assessment may assist in addressing other issues raised in the Science and Decisions (NRC 2009) report, such as addressing variability and biological understanding of the likely mode of action. Endogenous formaldehyde has been reported to be present in the human blood at concentrations ranging from 13 to 100 µM reported (Heck and Casanova 2004; IARC 2006). There are polymorphisms in the metabolic pathways involved in the metabolism of formaldehyde that may contribute to variability in endogenous concentrations observed across populations. In addition, reactive aldehydes have been demonstrated to cause leukemia in mice deficient in selected genes or isoforms of aldehyde dehydrogenase (Ridpath et al. 2007; Parmar and D'Andrea 2012; Garaycoechea et al. 2012; Rosado et al. 2011; Langevin et al. 2011). This research is demonstrating the potential for endogenous aldehydes to damage hematopoietic stem cells if either Fanconi Anemia genes and/or ALDH2 or ALD5 genes are knocked out. Under such conditions, mice spontaneously develop leukemia with no external exposure to chemicals (Swenberg et al. 2013). These types of results may be relevant to understanding the impact of individual and cell type variability in characterizing the shape of the dose-response curve in the low concentration region.

METHODS

Quantifying Endogenous Levels

Measuring concentrations of formaldehyde resulting from endogenous production versus exogenous exposure is a challenge, especially since formaldehyde is a reactive compound. As noted, endogenous formaldehyde can be present in concentrations ranging up to 100 µM, and these concentrations include measured formaldehyde contained in formaldehyde DNA-adducts. Formaldehyde may induce DNA adducts including N2-hydroxymethyl-deoxyguanosine (dG), N6-hydroxymethyl-deoxyadenosine(dA), and N4-hydroxymethyl-deoxycytosine(dC) *in vitro*, with measurement of concentrations of dG

adducts following inhaled formaldehyde exposure reported by Swenberg and colleagues (Lu et al. 2011, 2012, Moeller et al. 2011).

Generally, adducts can be used as biomarkers of exposure, as well as key events in the development of adverse endpoints, such as cancer. DNA adducts have been used as molecular dosimeters to better reflect the internal dose of a genotoxic chemical in target tissues following exposure. However, quantifying the number of adducts resulting from exogenous exposure to formaldehyde has proven difficult because of the substantial natural endogenous background of formaldehyde adducts.

Recent studies in both rats and nonhuman primates employing stable isotope-labeled formaldehyde afford the ability to differentiate between formaldehyde adducts of endogenous and exogenous origin (Lu et al. 2010a; Lu et al., 2010b; Moeller et al., 2011). These studies employed [13CD2]-formaldehyde for exposure, coupled with highly sensitive mass spectrometry detection methods which allow for the differentiation between and separate quantitation of DNA adducts originating from endogenous and exogenous sources. This method has an extremely low limit of detection (LOD) (20 attomoles) and is consistent with standard approaches for the measurement of DNA damage and repair that employ LC-MS/MS.

Initial studies measured both endogenous and exogenous levels of dG and dA adducts following inhalation exposure of rats to 10 ppm formaldehyde (Lu et al. 2010). While endogenous levels of both adducts were present in all tissues (including nose, lung, liver, spleen, bone marrow, thymus, and blood), no exogenous dA adducts have been measured in any tissue following 1 or 5 days of exposure to 10 ppm formaldehyde in rats. Exogenous dG adducts were measured in the upper respiratory tract of these animals following inhalation exposure, with no exogenous dG adducts measured at sites distant from the portal of entry. Based on these initial results, dG adducts have been focused on to characterize potential exposure of tissues to exogenous formaldehyde. Additional studies have been conducted in nonhuman primates (Moeller et al. 2011) and follow on studies in the rat have evaluated adducts following exposures for up to 28 days (Swenberg et al. unpublished).

While formaldehyde DNA hydroxymethyl adducts are relatively unstable, if they are reduced with sodium cyanoborohydrate to a methyl group, they are stable and the

potential for artifactual DNA damage is minimal. The methods applied also minimized the loss of DNA adducts. The tissues were snap-frozen and stored at -80°C, with DNA isolation performed quickly and samples placed on ice. Following this, the DNA was reduced to convert OHMedG to MeddG, a stable DNA adduct, prior to hydrolysis. Internal standards were added and DNA nucleosides and adducts were enriched with HPLC and fraction collection. This approach results in chemical specific quantitation of exogenous and endogenous adducts. The adducts are clearly differentiated by mass spectrometry and the use of stable isotope exposures. These studies have also shown significant differences in endogenous formaldehyde dG adducts between tissues, with primate bone marrow having much higher amounts than all other tissues.

Similar results have been obtained for acetaldehyde (Moeller et al, 2013) and vinyl chloride through the use of stable isotope exposures (Mutlu et al, 2012).

Incorporation of Endogenous Levels into Dose-Response Modeling

Starr and Swenberg (2013) conducted an initial dose-response analysis that incorporates the endogenous production of formaldehyde DNA adducts. It is a novel "bottom-up" approach to the bounding of low-dose human cancer risks from chemical exposures and it does not rely upon high-dose data to develop an upper bound on low-dose cancer risk. The approach is consistent with the ''additivity to background'' concept and yields central and upper-bound risk estimates that are linear at all doses. In addition, it requires only information regarding background risk, background (endogenous) exposure, and the additional exogenous exposure of interest in order to be implemented.

This method provides an independent "reality check" on extrapolations from high-dose data and allows for extrapolation upward from background (endogenous) exposure and response, as opposed to the typical ''top-down'' approach that often requires downward extrapolation from exogenous exposure levels so extreme as to be potentially irrelevant to the true risks that might be present at the far lower environmental exposures that are of primary interest. Figure 1 provides the key elements of the approach, with P_0 representing the background lifetime risk of a tissue-specific cancer in humans (Starr and Swenberg 2013). C_0 represents the tissue-specific background steady-

state concentration of a biomarker presumed to be causally related to this cancer, such as a DNA adduct. Then the ratio P_0/C_0 provides an estimate of the low-dose slope of the relationships between the cancer risk and the corresponding site-specific DNA adduct concentration. Similarly, if C_{0L} represents the *lower* 95% confidence bound estimate for the same background adduct concentration, then the ratio P_0/C_{0L} provides an *upper* 95% confidence bound on the low-dose slope. This latter ratio is thus directly comparable to the q_1* derived from high dose animal studies, as well as the upper bound slope estimates for the low-dose linear dose-response relationships that are typically inferred from epidemiologic analyses of occupational cohort cancer mortality, provided only that the dose metrics used in these two kinds of studies (animal bioassays and cohort mortality studies) are converted into the corresponding equivalent site-specific adduct concentrations.

Impact of Endogenous Levels on Target Tissue Dosimetry

A BBDR model has been developed for formaldehyde (Conolly et al. 2003, 2004) and has been applied in dose-response modeling in the most recent EPA (2010) Toxicological Review of Formaldehyde – Inhalation Assessment. In reviewing this assessment the National Academy of Sciences (NRC 2011) noted that:

> *"Given that the BBDR model for formaldehyde is one of the best-developed BBDR models to date, the positive attributes of BBDR models generally, and the limitations of human data, the committee recommends that EPA use the BBDR model for formaldehyde…"*

The key elements involved in the development of these models were based on the available information on tissue dosimetry and mode of action for nasopharyngeal and lung cancer and included:

- three-dimensional computer reconstructions of the rat, monkey, and human nasal passages and computational fluid dynamics (CFD) modeling to predict regional dosimetry of formaldehyde;
- association of the flux of formaldehyde into the nasal mucosa, as predicted by the CFD model, with formation of DNA-protein cross-links (DPX) and with cytolethality/regenerative cellular proliferation (CRCP); and
- a two-stage clonal growth model to link DPX and CRCP with tumor formation (Conolly et al. 2003, 2004).

The computational fluid dynamics (CFD) models and lung dosimetry models used to characterize the absorption of formaldehyde from the nasal and lung cavities (Kimbell et al. 2001; Overton et al. 2001) did not account for the presence of endogenous formaldehyde. The mass transfer coefficients governing the rate of formaldehyde absorption on respiratory airway walls were calibrated to nasal uptake data in rats exposed to high concentrations of formaldehyde. There was insufficient data to calibrate the boundary conditions at lower formaldehyde exposure concentrations and to include the effects of endogenous formaldehyde.

Initial CFD modeling has been conducted to investigate the impact of the presence of endogenous formaldehyde on the absorption of exogenous formaldehyde from the nasal cavity of rats, monkeys, and humans. In this effort (Schroeter et al., 2013), the boundary conditions in the nasal CFD models were modified to include formaldehyde air:tissue partitioning, saturable metabolism, first-order clearance, DNA binding, and endogenous production. Using this approach, formaldehyde absorption in the upper respiratory tract was simulated according to its pharmacokinetic description in nasal tissues, including the presence of endogenous formaldehyde. Updated flux values were computed at regions in the rat and monkey nasal passages where DPX and cell proliferation rates were measured for inclusion into the BBDR models.

RESULTS

Quantifying Endogenous Levels

The results from the Lu et al. (2010, 2011) studies in rats and the Moeller et al. (2011) study in nonhuman primates, provide a comparison of the endogenously present formaldehyde levels versus those resulting from exogenous exposure (Figure 2).

Results from a recent 28-day rat study (Swenberg, unpublished) provide data regarding the time needed for dG formaldehyde adducts in the nasal turbinates to come to steady state following inhalation exposure to formaldehyde. Compared to a single 6 hour exposure to 2 ppm [13CD2]-formaldehyde (6 hr/day, 7 days/week), the 28 day study demonstrated steady state concentrations of adducts that are 5.6-fold greater than a single exposure. This is important, as endogenous adducts are expected to be at steady-state concentrations (unpublished data).

As noted previously, the studies conducted to date have demonstrated endogenous dG adducts in all tissues examined, but have only found exogenous dG adducts in nasal tissue following inhalation exposure to formaldehyde. This suggests that formaldehyde did not reach the circulating blood in an active form. Research conducted with other biomarkers, including hemoglobin adducts and formyllysine adducts, is consistent with these results. In rats exposed to 2 ppm formaldehyde 6 hours/day for up to 4 weeks (Andrews-Kingon et al. 2013), hemoglobin adducts, specifically imidazolidone formation on the N-terminal valine, were measured. No exogenous hemoglobin-formaldehyde (Hb-FA) adducts were detected in any of the samples following exposure for 1 day to 4 weeks. Endogenous Hb-FA adducts averaged 12.7±3.7 pmoles/mg Hb and 15.6±2.3 pmoles/mg Hb for the 1 and 5 day exposure samples, respectively. Even with increased duration of exposure, no exogenous adducts were detected in RBCs, while endogenous levels were >500x above the limit of detection.

An additional study (Edrissi et al. 2013), measuredN6-formyllysine adducts in several tissues from rats exposed to 0.7, 2, 6 or 9.1 ppm [13CD2]-formaldehyde for 6 hr/day for 28 days. Exposure-related stable isotope adducts were present in nasal turbinates. Further analysis are ongoing to determine the rates of formation and the loss of N6-formyllysine over a 7 day post exposure period. Endogenous formaldehyde is a

source of lysine N6-formylation and this adduct is widespread among proteins in all cellular compartments. Consistent with the previous studies, endogenous adducts were present in all tissues examined, but exogenous N6-formyllysine adducts were only detected in nasal epithelium of rats exposed to [13C2H2]-formaldehyde by inhalation, but not in lung and liver.

Research is ongoing to conduct similar studies for other compounds (e.g, methanol) that may not necessarily be endogenously present, but may be metabolized to endogenously present compounds (Lu et al. 2012). The carcinogenic potential of methanol has been debated, because it is metabolized to formaldehyde. Studies were conducted in rats exposure daily to [$^{18}CD_4$]-methanol by gavage (500 or 2000 mg/kg) for up to 5 days and quantification of formaldehyde-specific endogenous and exogenous DNA adducts measured. The results demonstrated that labeled formaldehyde arising from [$^{13}CD_4$]-methanol induced hydroxymethyl DNA adducts in multiple tissues in a dose-dependent manner. The results also demonstrated that the number of exogenous DNA adducts was lower than the number of endogenous hydroxymethyl DNA adducts in all tissues of rats administered 500 mg/kg per day for 5 days, a lethal dose to humans. Endogenous dA formaldehyde adducts were present in all tissues, while exogenous dA adducts were only detected when formaldehyde was formed intracellularly from metabolism, as shown for bone marrow and kidney for methanol (Lu et al, 2012).

Incorporation of Endogenous Adduct Data into Dose-Response Assessment

Figure 3 provides the mean and 95% lower confidence bound (Starr and Swenberg et al. 2013) on the number of endogenous and exogenous dG adducts per 10^7dG in nasal respiratory epithelium and bone marrow as determined in monkeys following two 6 hour exposures to 2 ppm [13CD2]-formaldehyde (Swenberg et al., 2011). Also provided are the corresponding steady-state exogenous dG adduct levels that would result from continuous 24 h/day, 7 days/week exposure (Starr and Swenberg 2013). To estimate the exogenous levels associated with continuous exposure, the adduct levels measured in monkeys by Moeller et al. (2011) immediately after the two 6 h exposures (30 h after the onset of the first exposure), together with a simple one compartment linear kinetic model of adduct buildup and elimination with a 63 h

elimination half-life (mean adduct lifetime T = 63/ln(2) = 90.9 h) as has been determined in rats (Swenberg et al., 2013) was applied.

The results of the "bottom up" approach, which relies upon the estimated exogenous adduct levels at steady state indicate:

- For nasopharyngeal cancer: the bottom-up UCL95 risk estimate is 29.8-fold lower than the draft USEPA (2010) top-down estimate of 1.1%

- For lymphohematopoietic/leukemias: based on the detection limit for DNA adducts, the bottom-up UCL95 risk estimate is at least 14,615-fold lower than the draft USEPA (2010) top-down estimate of 5.7%

These large discrepancies suggest that the top-down approach may be overly conservative. The true dose-response relationship may well be highly nonlinear, with far smaller risks occurring at low doses that are predicted by a linear dose-response relationship. As exogenous exposure increases from zero, at some point nonlinear processes are likely to begin influencing the carcinogenic response, leading to greater than linear risk increments. For this reason, the bottom-up approach may not be appropriate for bounding risks in the observable response range, where the dose-response relationship for tumor incidence may be highly nonlinear due to factors such as cytotoxicity, tissue damage, and enhanced cell proliferation. The doses are which these factors are expected to be critical can only be determined through a comprehensive and deep mechanistic understanding of how chemical exposures give rise to human cancer.

Impact of Endogenous Levels on Target Tissue Dosimetry

The CFD models were used to simulate nasal uptake of inhaled formaldehyde in the presence of endogenous formaldehyde in rats, monkeys, and humans. Exposure concentrations ranged from 1 ppb to over 10 ppm. At exposure concentrations ≥ 1 ppm, predicted nasal uptake was very high, in agreement with past studies (Figure 4). Endogenous formaldehyde had no effect on nasal uptake at exposure concentrations > 500 ppb. However, the presence of endogenous formaldehyde reduced nasal tissue dose of inhaled exogenous formaldehyde at lower exposure concentrations, most notably at concentrations < 10 ppb (Figure 4). Tissue dose was greatly reduced at exposure concentrations in the low ppb range. At high exposure concentrations, formaldehyde concentrations are much greater in the air than in the tissue, which leads to rapid

absorption in the anterior nasal passages due to the high rate of formaldehyde partitioning into nasal tissues. At low exposure concentrations, the concentration gradient between air and tissue is greatly reduced due to the presence of endogenous formaldehyde in nasal tissues, leading to reduced tissue dose. These results suggest that understanding endogenous concentrations of a compound such as formaldehyde are of critical importance in characterizing the shape of the dose response curve in the low dose region.

DISCUSSION

The research centered on the characterization of endogenous concentrations of formaldehyde for this case study has focused on specific adducts as a biomarker of exposure. However, there is limited current research that demonstrates the relationship between the formaldehyde-DNA adducts (crosslinks) and tumors. The adducts were not considered a biomarker of effect or necessarily being causally related to tumors.

The adducts focused upon as a biomarker of exposure for inhalation exposure to formaldehyde (dG adducts) are considered to be mildly pro-mutagenic (not potent) and a key event in the initiation of mutations that lead to carcinogenesis. Moeller et al. (2013) has presented preliminary data to suggest that some of the dG adducts may be breakdown products of DNA-protein crosslinks (DPX), which are considered as key events in understanding the mode of action for potential carcinogens (USEPA 2010). Preliminary qualitative data suggest that DPX may be breaking down to the mono-adduct and ultimately to dG. There is still remaining research to be conducted to evaluate this potential connection, because chemical-specific DPX methods have not been developed to quantify these initial qualitative results. Although it is recognized that not every adduct leads to a mutation that leads to tumors, the initial BBDR and "bottom up" approaches made the conservative assumption that the adducts were quantitatively related to tumor development.

The bottom up approach" also assumes that formaldehyde plays a causal role in leukemia risk, and that the development of all relevant leukemias is associated with or results from adduct formation. The bottom up approach uses the adduct levels from endogenous exposure as the relevant dose metric to account for background risk. As discussed above, there is qualitative data to support this association, but there is

additional research needed to support the direct association. Additional investigation into the relevance of endogenous adducts to the development of disease is also needed, since the body cannot distinguish between adducts related to endogenous and exogenous exposure. The body may treat exogenous and endogenously formed formaldehyde differently, but once the adduct is formed, there should be little difference in interactions within tissue or cells. For formaldehyde and other reactive aldehydes, investigations are ongoing to determine the impact on disease states in animals (Ridpath et al. 2007; Parmar and Andrea 2012; Garaycoechea et al. 2012; Rosado et al. 2011; Langevin et al. 2011). Initial results suggest that endogenous aldehydes may contribute to selected diseases only in animals deficient in selected genes or isoforms of aldehyde dehydrogenase, critical for the metabolism of the reactive aldehyde. Determining the impact of the deficiencies in animals and human may contribute to the further understanding of variability in response in humans.

In the application of the bottom up approach, the shape of the dose-response curve for exogenous exposure is assumed to be linear. If the "true" dose response is highly nonlinear even in the low exogenous dose range (i.e., well below the observable response range), then the bottom-up approach will produce an upper bound on low dose risk that may well exceed the true risk by orders or magnitude. This is not qualitatively different from what occurs in these circumstances with upper bounds developed with the top-down approach.

The important quantitative difference between the two approaches arises from the smaller uncertainties of the background risk and background exposure levels in comparison to those found at the low end of and within the observable response range. In the case of formaldehyde, the human lifetime background risks of nasopharyngeal cancer, Hodgkin lymphoma, and leukemia are known with far greater certainty than are the incremental risks of these cancers in occupationally exposed workers. Furthermore, the target tissues associated with these cancers are known with far greater certainty (assuming monkeys to be a valid surrogate species for humans) than are the incremental exogenous exposure levels arising from occupational exposure. These two factors lead to the far tighter upper confidence bounds on low-dose human cancer risk that are associated with the bottom-up approach. However, it is important to note that the

available results from CFD modeling may provide accurate estimates of the tissue dosimetry associated with occupational exposures and are not incorporated into the bottom up approach.

EPA (2013) recently presented results from a new analysis of the rat nasal tumor data from the CIIT studies (Kerns et al. 1983, Monticello et al. 1996), using background endogenous and exogenous dG adduct data obtained from rat nasal tissues following a single 6 hour exposure to $^{13}CD_2$ formaldehyde. A "modified" Weibull dose-response model (forced to include a linear term) was fit to the tumor data versus total adduct concentration. While no details regarding the fitting process were provided, the resulting estimated risk at low exogenous exposures from this alternative approach exceeded the upper bound risk estimates that would be obtained using the bottom-up approach of Starr and Swenberg (2013), suggesting the bottom-up approach would *never* overestimate risk at low doses.

While this provides an alternative approach, this approach raises additional issues. First, the "modified" Weibull model does not provide central estimates of risk. Rather, it generates bounding risk estimates that are constrained to be linear at low doses. It is not clear what the appropriate best-fitting dose-response model is for this case, but a pure Weibull model produces low-dose risk estimates that are far lower than those arising from this "modified" Weibull model. As an alternative to the "modified" Weibull model, EPA also attempted to apply a conventional multi-stage model analysis of the tumor data versus dG adduct concentration; however, the BMDS software package used by the EPA in the application of these models failed to achieve convergence on an upper bound for the low dose risks. While additional analyses are current underway to further investigate and resolve these questions, at this time a conventional "top-down" multi-stage model analysis of the rat tumor data versus airborne formaldehyde concentration yields a very highly nonlinear central estimate of risk at low doses, and the bottom-up approach yields an upper bound risk estimate that is markedly higher than the conventional top-down central estimate.

In the case of an endogenously present compound such as formaldehyde, the presence of the "background" concentration may give the appearance of a threshold, with low concentrations of exogenous exposure not contributing significantly to the

endogenous concentrations present. However, in the case of formaldehyde, the proposed mode of action for the tumors of interest in the upper respiratory tract suggests that high concentrations associated with cytotoxicity are necessary for the development of a carcinogenic response. In the animal bioassay conducted in rats and mice (Kerns et al. 1983, Swenberg et al. 1980; Monticello et al. 1996), the lowest concentration where nasal tumors were seen in the animal bioassay (6 ppm) is also cytotoxic. The current BBDR model for formaldehyde (Conolly et al. 2003, 2004) includes a low-dose linear component based on the DNA-protein adducts, as well as consideration of cytotoxicity. In addition, there are probably more than two components to the dose-response curve, since each of the steps of adduct formation, mutation, cytotoxicity, etc., would have its own dose-response. The BBDR is consistent with the results of a HESI (Health and Environmental Sciences Institute) project addressing the relationship between adducts and cancer, which recommended breaking down each part of the process (Jarabek et al., 2009; Himmelstein et al., 2009).

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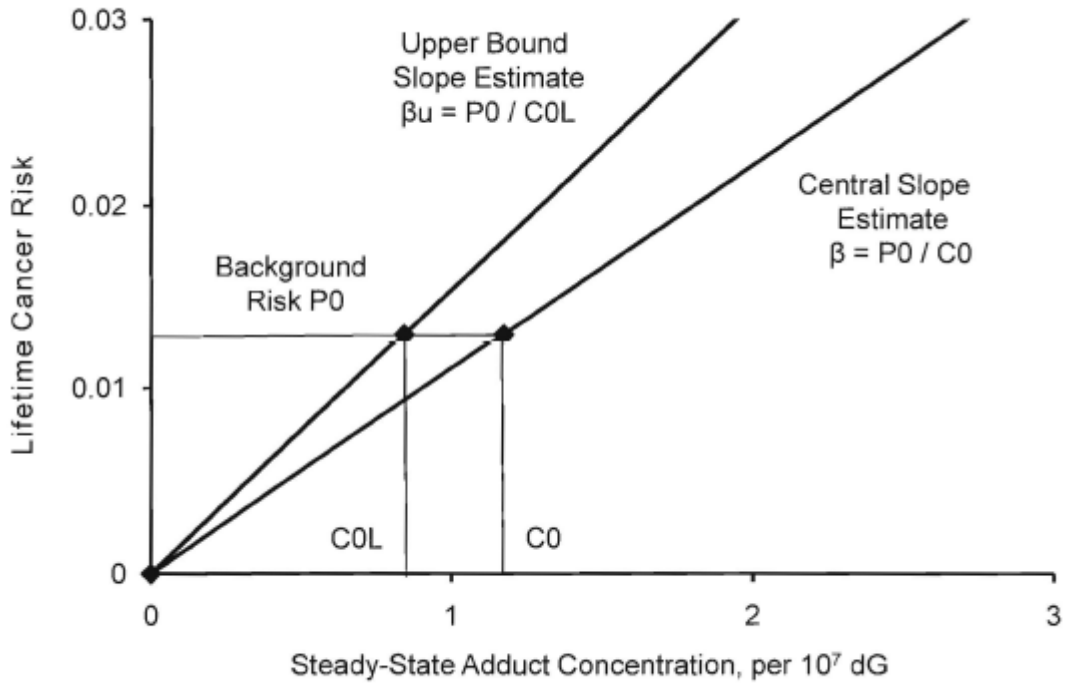
Figure 1. "Bottom Up" Approach



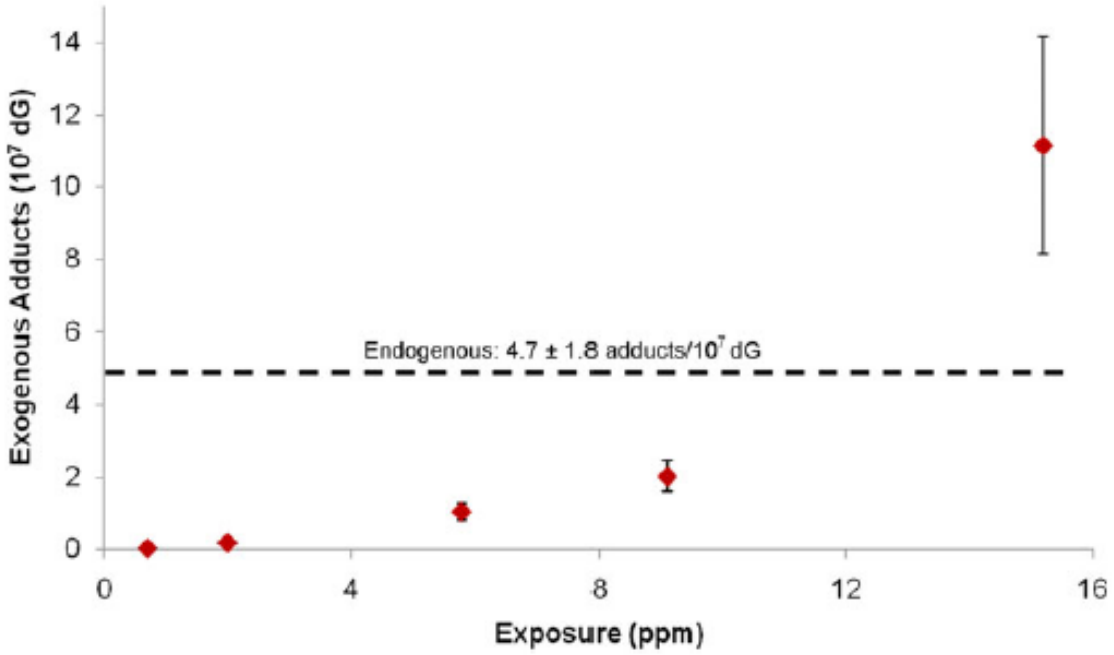


FIG. 2. Molecular dosimetry of N^2-hydroxymethyl-dG adducts in rats exposed to formaldehyde.

From Swenberg et al. (2011)

Tissue	Endogenous Adducts at 30 hrs	Exogenous Adducts at 30 hrs	Exogenous Adducts at Steady-State
Nasal Epithelium Mean ± se Lower 95% Bound	 2.49 ± 0.23 2.11	 0.25 ± 0.020	 2.21 ± 0.18
Bone Marrow Mean ± se Lower 95% Bound	 17.5 ± 1.31 15.34	 < 0.00103[a]	 <0.00912[a]

a: no exogenous adducts were detected in bone marrow; upper limit estimate based on the detection limit reported in Moeller et al. (2011).

Figure 3: N2-hydroxymethyl-dG Adducts in Monkeys Exposed Twice for 6 hrs to 2 ppm CH2O

Exposure Concentration (ppm)	Nasal Uptake (%)		
	Rat	Monkey	Human
1.0	99.4	86.5	85.3
0.1	98.6	86.5	84.7
0.01	91.3	84.1	77.1
0.001	17.5	42.8	n/a[1]

[1]the predicted formaldehyde concentration at the model outlet was greater than the exposure concentration, indicating net desorption of formaldehyde vapor

Figure 4: Predicted nasal uptake in the rat, monkey, and human nasal passages using the CFD models incorporating endogenous formaldehyde. From Schroeter et al. (2013).