Case Study–Endogenous Chemical Risk Assessment: Formaldehyde as a Case Example

1. **Summary of Method Illustrated by Case Study.**

An understanding of the effects of background processes or endogenous concentrations is important in characterizing the shape of the dose-response curve in the low-dose region (e.g., linear versus nonlinear) for endogenously present compounds. Measuring concentrations of formaldehyde resulting from endogenous production versus exogenous exposure is a challenge, especially since formaldehyde is a reactive compound. However, recent studies in both rats and nonhuman primates employing stable isotope-labeled formaldehyde have differentiated between formaldehyde DNA adducts of endogenous and exogenous origin (Lu et al. 2011, 2012, Moeller et al. 2011). DNA adducts have been used as molecular dosimeters to reflect the internal dose of a genotoxic chemical in target tissues following exposure. These studies employed [13CD2]-formaldehyde for exogenous exposure, coupled with highly sensitive mass spectrometry detection methods. The results from these studies provide an alternate characterization of exposure that can be incorporated into dose-response assessments for the potential carcinogenicity of formaldehyde. The purpose of this case study is to discuss endogenous and exogenous formaldehyde DNA adducts and their application in two risk assessment approaches that accommodate endogenous production of formaldehyde: 1) a "bottom up" approach; and 2) a biologically-based dose-response (BBDR) model.[1]

Empirical dose-response modeling, such as with empirical Weibull or multistage models, are based on statistical fits to the tumor dose-response in the observable range. These models are then used to extrapolate downward to environmentally relevant external exposure concentrations. The "bottom up" approach uses a simple linear model (Starr and Swenberg 2013) that can be extrapolated upward from background (endogenous) exposure and response levels, rather than downward from the observable response range. The approach is consistent with the ''additivity to background'' concept and yields both 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. In the case of formaldehyde, the bottom-up approach uses DNA adduct levels arising from endogenous formaldehyde as the relevant dose metric to account for background risk.

In addition, the case study team is currently working on refining the target tissue dosimetry component of the formaldehyde biologically-based dose-response (BBDR) model to include a description of endogenous formaldehyde and characterize its impact on tissue uptake of exogenous formaldehyde (Schroeter et al. 2013). This revised characterization of target tissue

[1] BBDR models for formaldehyde include computational fluid dynamic (CFD)-generated predictions of the regional flux of formaldehyde into tissues and parameters that are linked to two modes of action proposed for tumor development. These modes of action are described by parameters of a two-stage clonal growth model, which describes cancer as a succession of genetic changes and altered growth behaviors that lead to progressive conversion of normal cells into cancer cells. While the clonal growth model may not be an accurate representation of the actual cellular mechanism of formaldehyde carcinogenesis, it does provide insight into the relative importance of direct mutagenicity and cellular proliferation related to cytotoxicity in tumor development at high exogenous doses.

dosimetry can be incorporated into the full BBDR models (Conolly et al. 2003, 2004) to characterize a range of plausible risk estimates.

2. **Describe the problem formulation(s) the case study is designed to address. How is the method described in the case useful for addressing the problem formulation?**

Conducting a risk assessment for a compound that is present endogenously poses several challenges. First, methods are needed to quantify endogenous production and differentiate DNA damage arising due to endogenous production from biochemically 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 best interpret the results and incorporate those results into an appropriate dose-response assessment. The risk assessor must also attempt to determine whether exogenous exposures can increase the tissue levels sufficiently 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, off-gassing from building materials, and tobacco smoke; it arises as well as from the metabolism of foods, chemicals and drugs. 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 the use of recent research on specific formaldehyde DNA adducts to characterize biomarkers of exposure for both endogenous and exogenous formaldehyde. The application of this information into two methods for estimating the dose-response curve (bottom up and BBDR) and the potential impact on the shape of the dose-response curve in the low concentration region are also discussed.

3. **Comment on whether the method is general enough to be used directly, or if it can be extrapolated, for application to other chemicals and/or problem formulations. Please explain why or why not.**

The approaches in this case study are not specific to formaldehyde. They can be extended to other compounds that may or may not be endogenously present. Initial work is underway to extend these methods to other endogenous compounds that produce DNA adducts such as acetaldehyde, ethylene oxide and vinyl chloride. The two approaches in this case study in which endogenous production has been accommodated (bottom-up approach and a component of the BBDR model) demonstrate the challenges that exist in collecting the appropriate information

needed to characterize the dose-response curve in the low-concentration range, which is of great practical significance in estimating and bounding risks from ambient exposures. The DNA adducts relied upon in the case study may also be indicative of general phenomena related to endogenous DNA damage.

4. **Discuss the overall strengths and limitations of the methodology.**

Strengths:

- Use of biomarkers, such as specific DNA adducts, which are closer to the critical "target tissue" concentrations than is the corresponding external exposure concentration.
- Reliance on a highly sensitive and accurate method that differentiates between exogenous and endogenous concentrations.
- Approaches for the measurement of exposure biomarkers and application of the "bottom up" approach can be extended to other compounds.
- CFD modeling has been conducted to investigate the impact of the presence of endogenous formaldehyde on the site-specific absorption of exogenous formaldehyde in the nasal cavities of rats, monkeys, and humans.

Limitations:

- Reliance of the "bottom-up" approach on the assumption of a linear dose-response relationship restricts it to bounding low-dose cancer risks; it may not be appropriate for bounding risks in the observable response range, where nonlinear processes can dominate the dose-response relationship, or for developing "best" or central estimates of risks.
- Pharmacokinetic assumptions are required to convert the quantified biomarkers of exposure (DNA adducts) that are obtained in short-term animal studies to corresponding estimates arising from continuous lifetime exposures in humans.
- Potential variability in the endogenous concentrations present in humans has not yet been quantified, although interanimal variation in endogenous concentrations has been quantified and this variation has been employed explicitly in developing lower bound estimates of background endogenous concentrations in different tissues and species.

5. **Outline the minimum data requirements and describe the types of data needed.**

- Biomarkers of exposure that are plausibly linked to either the noncarcinogenic or carcinogenic process to characterize the endogenously present concentrations, as well as the contributions arising from exogenous exposure.
- PK and, possibly, BBDR models to characterize the target tissue dosimetry associated with endogenous and exogenous exposure.
- Incorporation of data into the 'bottom up" approach and interpretation of results.

How this assessment addresses issues raised in Science & Decisions:

- **B. Address background exposures and responses?** This case study demonstrates for an endogenously present compound the impact of endogenous and exogenous exposure on target tissue dosimetry and upper bounds on the dose-response curve in the low concentration region.
- **G. Work practically?** The bottom up approach is a relatively easy method to apply, as long as the critical data are available.

References Cited:

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2003. Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicological Sciences 75(2):432-437.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ. 2004. Human respiratory tract cancer risks of inhaled formaldehyde: does-response predictions dervied from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicological Sciences 82(1):279-296.

Lu K, Moeller B, Doyle-Eisele M, McDonald J, Swenberg J. 2011. Molecular dosimetry of N2-hydroxymethyl-dG DNA adducts in rats exposed to formaldehyde. Chem Res Toxicol 24(2), 159-61.

Lu K, Craft S, Nakamura J, Moeller BC, Swenberg JA. 2012. Use of LC-MS/MS and stable isotopes to differentiate hydroxymethyl and methyl DNA adducts from formaldehyde and nitrosodimethylamine. Chem Res Toxicol 25(3):664-75.

Moeller B, Lu K, Doyle-Eisele M, McDonald J, Gigliotti A, Swenberg J. 2011. Determination of N^2-Hydroxymethyl-dG Adducts in the Nasal Epithelium and Bone marrow of Nonhuman Primates Following $^{13}CD_2$-Formaldehyde Inhalation Exposure. Chem Res Toxicol 24: 162-164.

Schroeter JD, Campbell J, Kimbell JS, Conolly RB, Clewell HJ, Andersen ME. 2013. Effects of endogenous formaldehyde in nasal tissues on inhaled formaldehyde dosimetry predictions in the rat, monkey, and human nasal passages. Currently in preparation.

Starr TB, Swenberg JA. 2013. A novel bottom-up approach to bounding low-dose cancer risks from chemical exposures. Regulatory Toxicology and Pharmacology 65(3):311-315.