ARA Workshop
February 15-17
Institute for the Advancement of Food and Nutrition Sciences, Washington, DC

Case Study
Use of Molecular Dosimetry to Identify Points of Departure for Potential Carcinogens with Both Endogenous and Exogenous Exposures

Sponsor: ACC Formaldehyde Panel
Speakers: J. Sherman, K. Lu, R. Schoeny, R. Conolly

Introduction

This research case study describes use of rapidly developing and highly sensitive LC MS/MS technologies, such as Stable Isotope Labeling and Mass Spectrometry (SILMS). We are seeking expert opinions on how SILMS generated dosimetry data for molecular targets (DNA and protein) in target tissues can be used for potentially mutagenic and/or carcinogenic chemicals to reliably quantitate risk at the lower end of the dose-response curve. The overall goal is to characterize the shape of the cancer dose-response curve. Due to insufficiently sensitive methods used to date, neither animal studies, mutation assays or analytical techniques alone have provided data useful in determining shape of the dose-response curve for either mutagenicity or tumorigenic response at low – human relevant – levels of exposure. This frequently necessitated the use of default assumptions, often that, for potentially mutagenic chemicals, that the mode of action (MOA) for cancer depended on an early mutagenic key event. Most often this carried a corollary assumption of a linear low dose response. Recent publications demonstrate that SILMS can provide highly reliable molecular dosimetry data at a molecular target (i.e., DNA) in relevant target tissues at very low levels of exposure. Additionally, for some chemicals, SILMS data have conclusively demonstrated that exposure of the DNA in target tissues is highly non-linear. Thus, these new analytical methodologies provide direct evidence that for some potentially mutagenic chemicals, a linear cancer process involving mutation is not happening at low levels of exposure. Importantly, the new LC MS/MS tools can differentiate DNA exposure to endogenously produced chemicals from exogenous exposure to the same chemicals. As such, this data enables reliable quantification of relative risk from endogenous versus exogenous exposures.

The purpose of this research case study is to assess the use of molecular dosimetry for chemicals with both endogenous and exogenous exposures and to provide recommendations for improved integration of molecular dosimetry into cancer risk assessments. The emphasis is on identifying points of departure along a continuous dose-response curve; that is the point at which exogenous exposures are sufficiently below endogenous exposures that they become biologically insignificant and/or pose *de minimis* risk.

US EPA's *Guidelines for Carcinogen Risk Assessment* (Cancer Guidelines) (US EPA, 2005) support this case study, as they state that a dose-response assessment based on precursor data such as DNA adducts is supported. For example, the Cancer Guidelines state:

> "Cancer is a collection of several diseases that develop through cell and tissue changes over time. Dose-response assessment procedures based on tumor incidence have seldom taken into account the effects of key precursor events within the whole biological process due to lack of empirical data and understanding about these events. In this discussion, response data include measures of key precursor events considered integral to the carcinogenic process in addition to tumor incidence. These responses may include changes in DNA, chromosomes, or other key macromolecules; effects on growth signal transduction, including induction of hormonal changes; or physiological or toxic effects that include proliferative events diagnosed as precancerous but not pathology that is judged to be cancer. Analysis of such responses may be done along with that of tumor incidence to enhance the tumor dose-response analysis. ***If dose response analysis of nontumor key events is more informative about the carcinogenic process for an agent, it can be used in lieu of,*** or in conjunction with, tumor incidence analysis for the overall dose-response assessment." (EPA, 2005, page 3-2).

This case study describes SILMS data for two potentially genotoxic chemicals that are also rodent nasal carcinogens, with both endogenous and exogenous exposures to either the chemical itself (formaldehyde) or to a proximal metabolite of the chemical of interest (acetaldehyde metabolite of vinyl acetate).

Since the formation of DNA adducts rat nasal epithelium is a continuous response, and no clear guidance on selecting a POD for a continuous response for DNA adducts is recognized, a consensus opinion would improve application of these emerging technologies in quantitative risk assessment.

1. Provide a few sentences summarizing the method illustrated by the case study.

Newly emerging ultrasensitive MS methods have enabled highly reliable quantification of molecular dosimetry to the DNA and, for the first time, allows direct characterization of the dose-response curve for DNA adduct formation throughout the range of potential exposures. Using molecular dosimetry data can obviate the need to characterize toxicokinetic and toxicodynamic influences on the shape of the dose-response curve at the putative molecular target(s), since the chemicals of interest have already been distributed and subjected to toxicodynamic influences, before reaching the molecular target in the tissue of interest. As such, the molecular dosimetry data can be directly applied with reduced uncertainties when performing quantitative risk evaluations. In addition, using stable isotope labeled test substances, SILMS can differentiate between DNA adducts produced from exogenous exposures from those due to endogenous exposures. One can then quantitate relative source contributions as well as combined exposures to both the relevant tissue(s) and potential molecular targets in those tissues. These considerations have been more fully described in two papers published in December, 2021: a review of the SLIMS (Lu et al., 2021) and a publication characterizing DNA adducts in potential target tissues and organs in rats after inhalation exposure to vinyl acetate (Hsiao, 2021).

For both formaldehyde and vinyl acetate, the relative contribution of endogenous and exogenous sources of formaldehyde and acetaldehyde in rat nasal epithelium was evaluated using SILMS after exposure concentrations spanning over 5 orders of magnitude. After repeated daily inhalation exposures to exogenously supplied heavy isotope labeled formaldehyde ([$^{13}CD_2$]-FA: 0.001 to 15 ppm) and vinyl acetate ([13C2]-VAM: 0.02-600 ppm),

nano-LC-ESI-MS/MS-SRM was used to quantify the most abundant DNA nucleotides adducted with formaldehyde and acetaldehyde. Additionally, DNA-protein cross links, measured as dG-Me-Cys adducts, were also evaluated after formaldehyde exposure. As described (Lu et al., 2021; Hsiao, 2021), due to recent advancements in this technology, reliable quantitation of N2-Et-dG adducts in various tissues, after vinyl acetate exposure, is now possible at fewer than 1 adducted dG per 1,000,000,000 non-adducted dG.

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?

The SILMS methods and the resulting data would be applicable to various problem formulations that used in regulatory or other risk assessment and risk management evaluations. Exposure scenarios for formaldehyde could include occupational settings (e.g., for manufacturing or other potentially high exposure scenarios such as formalin fixation and examination of fixed tissues). Lower exposure could be expected from off-gassing of manufactured materials (e.g. FEMA trailers, formaldehyde based resins and polymers). For any of these exposure scenarios, different conceptual models can be developed, and these may emphasize characterization of likely exposure, and the most likely MOA operable at exposures that are relevant to the adverse outcome of concern. Considering a variety of conceptual models for specific exposure conditions, we have identified several approaches for consideration during this case study, as follows:

1) Determine when the contribution of exogenous exposures to endogenous levels of DNA adducts to the total formaldehyde-associated adduct level (combined adducts) is no longer statistically significant (i.e., $p \leq 0.05$ or $p \leq 0.001$) from background. A conclusion would be that the risk is no longer biologically relevant and presents a *de minimis* risk
2) Determine when exogenous DNA adducts are less than or equal to a defined percentage of natural variability (e.g., ≤10% of one SD of the mean concentration of endogenous). A conclusion would be that the additional exposure is no longer biologically relevant and presents a *de minimis* risk
3) Determine a mean level of exogenous DNA adducts that reaches a defined percentage of the mean level endogenous adducts (e.g., 1/100 or $1/1000^{th}$ of the mean endogenous level). A conclusion would be that the exposures are no longer biologically relevant and present *de minimis* risk
4) Use a biologically-based modeling approach to estimate a continuous response for cancer risk in the low-dose region (e.g., BBDR modeling of outcome), then set a regulatory limit based on
    a. A defined acceptable risk (e.g., 10^{-6}), or
    b. A percentage of endogenous risk (e.g., background risk at 10^{-3} with acceptable increased risk $1/100^{th}$ of endogenous (i.e., 10^{-5})
5) Apply benchmark dose modeling with a defined response rate (e.g., BMD_{10}) for molecular dosimetry of DNA adducts.
6) Other suggestions from the Committee that would reflect best available science for integrating SILMS molecular dosimetry into quantitative risk assessment for potential genotoxic carcinogens with both endogenous and exogenous exposures

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.

This method is general enough to be used for all chemicals with endogenous and exogenous exposures that form DNA and/or protein adducts, although considerable chemical-specific method development (e.g., identify the adduct(s) to be quantitated, isolation techniques specific to those adducts, and MS methods with desired sensitivity) is required before performing a full dose-response study. The methodology would not require the use of heavy isotope labeled test material if there were no endogenous adducts to confound the accurate quantification of exogenous adducts.

Expanding the use of SILMS to more endogenous aldehydes with exogenous exposures would be prove useful in determining generalizability of the methods and identifying defined groupings of related chemical substances. Currently, the dose-response for only two small aldehydes has been characterized, and the available data indicate similar molecular dosimetry responses. For both formaldehyde and VAM (acetaldehyde adducts) there is a non-linear decrease in adducts that occurs at doses thought to be below the range of saturable metabolic and other removal processes (e.g., binding and excretion). Additionally, there is a non-linear increase in adduct levels at concentrations above the range of saturation of removal processes, which precedes the range of observable increases in tumor incidence.

4. Discuss the overall strengths and weaknesses of the method.

<u>Strengths</u>

- Overcomes weaknesses of historical technologies (e.g., ^{32}P postlabeling, immunoassays)
- Affords unequivocal identification and quantification of adducts from both exogenous and endogenous sources
- Highly reliable and accurate quantification of exposure to the molecular target(s) in the tissue(s) of interest
- Molecular dosimetry greatly reduces the need to characterize toxicokinetic- and toxicodynamic-related uncertainties, since the chemicals of interest have already been subjected to toxicokinetic and toxicodynamic influences before reaching the molecular target in the target tissue, before the key precursor event of molecular binding occurs
- When used for chemicals with both exogenous and endogenous exposures, affords a direct determination of relative dose that in turn supports relative quantification of additional mutagenic and carcinogenic potential.
- Outputs of SILMS can be directly used as inputs for biological modeling
- Continued improvement in the sensitivity and specificity of the methods is expected

<u>Weaknesses</u>

- Requires very sophisticated and expensive laboratory animal inhalation exposures to concentrations spanning multiple orders of magnitude, some with low ppb concentrations, over extended time periods
- Expensive and very limited availability of the required preparatory and analytical instrumentation
- Requires highly trained technical staff to isolate, prepare the biological samples and run the analytical equipment
- Does not address the rate of fixation of DNA adducts into mutations (rate of removal from DNA or repair, mis-repair and consequences).
- Extrapolation to other chemicals not possible until a more encompassing base set of chemical evaluations with this technology has been developed
- Little practical experience or specific guidance on regarding how to integrate molecular dosimetry data into quantitative risk evaluations (e.g., how to define a regulatory limit based on dosimetry at the putative molecular target)

5. Outline the minimum data requirements and describe the types of data sets that are needed.

The minimum dataset required includes quantitative evaluation of adducts in tissues from animals exposed to high concentrations in the range of concentrations producing a carcinogenic response (easily defined) to low concentrations (uncertainty related to defining a *de minimus* level may lead to inclusion of unnecessarily low dose groups in animal studies). Low concentrations are defined as those at which adducts either become biologically insignificant (currently no scientific consensus or regulatory guidance on this POD) or non-detectable (detection limit varies depending on quality of sample preparation and instrumentation, as well as the amount of DNA loaded onto column). For adequately characterizing variability, a sufficient number of animals to perform statistical analyses should be included as a minimum data requirement in the experimental design.

At a minimum, a single adduct, preferably the most abundant adduct, is analyzed to enable a wider range of detections within the range of potential exposures. Evaluating a wider range of adducts could be more informative, but may greatly increase animal use and costs, as often only a single adduct can be quantitated from each sample when DNA isolated from selected tissues is limited. For example, very limited amounts of nasal epithelium are available from each rat, but a relatively large amount of liver tissue is available from each rat. This exemplifies why study design and sample preparation procedures are specific to the tissue and adduct of interest.

Does your case study:

A. Describe the dose-response relationship in the dose range relevant to human exposure?

It is possible for humans to be exposed to formaldehyde, vinyl acetate, and acetaldehyde (directly or via metabolism of VAM to acetaldehyde) in the range of air concentrations that produced an increase in nasal tumors in rats. These concentrations (6-15 ppm for formaldehyde and 200-600 ppm for VAM) are below the saturation limit in air, and historical data indicate

these concentrations were sometimes reached in an industrial setting and/or in cases of accidental environmental releases. In the high dose range, these concentrations have been shown to produce a several fold increase in the adduct loads in rat and non-human primate nasal epithelia. At the current occupational exposure limit for formaldehyde (OSHA PEL of 0.75 ppm), exogenous DNA adducts are formed at concentrations well below the levels of endogenous adducts. At the current occupational exposure limit for VAM (OSHA PEL of 10 ppm) exogenous DNA adducts are non-detectable at a detection limit that is orders of magnitude lower than the endogenous levels of DNA adducts.

B. Address human variability and sensitive populations?

DNA adducts due to formaldehyde have not been quantitated in human nasal epithelium. Yu et al. (2015) measured nasal epithelial adducts in Sprague Dawley rats (2.0 ppm, 6 hr/day, 7 days) and Cynomolgus monkeys (1.9 ppm, 6 hr/day, 2 days). The exogenous adduct levels were 0.35 adducts/10^7dG and 0.26 adducts/10^7 dG in rats and monkeys, respectively. These numbers are not directly comparable, given the different exposure durations, but suggest similar levels of adduct formation for exposures at or near 2 ppm. Conolly et al. (2000) described dose-response modeling of DNA-protein cross-link data obtained with a methodology that is less specific than SLIMS. These older data were available for F344 rats and Rhesus monkeys. The crosslink model was extrapolated to humans. The predicted dose-responses were similar (within about a factor of 10), though with human adduct levels predicted to be lower than rat or monkey levels above about 1 ppm exogenous formaldehyde. A similar modeling effort for the SLIMS adduct data would require CFD modeling of the cynomolgus exposures, extension of the rat modeling of the SLIMS adduct data to the Cynomolgus data, and finally, extrapolation of that model to provide more reliable predictions of the human dose-response.

Several highly efficient metabolic and removal processes exist in all tissues for small endogenous aldehydes. Both formaldehyde and acetaldehyde are produced endogenously in all animals; thus, the pathways for metabolism and removal are highly conserved within and across species. For example, no polymorphisms for any of the aldehyde dehydrogenases, including formaldehyde dehydrogenase, are known in the human population.

Since nasal epithelial tissue at the point-of-contact is the tissue of interest, a potential difference in systemic distribution is not a meaningful consideration. Also, nasal tumors are rare in humans (approximately 2,000 cases/yr in the US population of ~330,000,000), and the primary causes of those tumors are generally believed to be related to viral (i.e., RSV) infections and exposure to wood dust (Chang et al., 2021; ACS, 2022). No susceptible human populations have been suggested or identified.

C. Address background exposures or responses?

The case study presented is focused toward quantifying background exposures and increasing our understanding of the relative contribution of background/endogenous exposures in relation to exogenous exposures when evaluating a single biological response.

D. Address incorporation of existing biological understanding of the likely mode of action?

Several different approaches have been taken to incorporate the biological understanding of molecular dosimetry into MOA analyses of the risk of nasal tumors. For example, an MOA analysis following the IPCS MOA Framework (McGregor et al. 2006; Thompson et al., 2020), a "bottom up" approach (Star and Swenberg, 2013, 2016), and a BBDR modeling approach (Conolly et al., 2003, 2004; Campbell et al. 2020) have been published. Some of these are discussed in more detail in a recently published review of SILMS technology (Lu et al. 2021), Each of these approaches incorporated the DNA and/or protein adduct molecular dosimetry results into their MOA analyses, albeit in different ways. Additionally, although not part of this case study, SILMS has been recently used to rule out MOAs for leukemia (Gentry et al. 2020), as SILMS data conclusively demonstrates that at air concentrations ≤15 ppm, formaldehyde is not distributed beyond the nasal epithelium.

The focus of this workshop is to discuss the strengths and weaknesses of different approaches to incorporating molecular dosimetry into quantitative risk analyses. This includes using molecular dosimetry to support and/or rule out MOAs, such as a linear non-threshold MOA for nasal tumors at relevant concentrations.

E. Address other extrapolations, if relevant – insufficient data, including duration extrapolations, interspecies extrapolation?

Currently, SILMS methods have mainly been limited to characterizing the shape of the dose-response curve for DNA and intracellular proteins exposure to various tissues in rats. However, a non-human primate study was also conducted with formaldehyde, and the results were consistent with the rat data, after accounting for known intraspecies differences in airway architecture.

Considerable method development work (e.g., adduct half-life analysis) was done for both formaldehyde and vinyl acetate to better understand the number of repeat dose exposures needed to attain steady state exogenous adduct levels, since endogenous levels are already at steady state. The method development work is required to ensure that steady state or near steady state adducts, due to exogenous exposures, are achieved before performing an quantitative analysis of the relative dosimetry for both endogenous and exogenous inhalation exposures.

F. Address uncertainty?

As detailed above, there are remaining uncertainties related to the dose-response for adduct formation for chemicals other than formaldehyde and vinyl acetate. As such, there would be considerable uncertainties related to extrapolating those results to other chemicals, including those with only exogenous exposures. There are only minor uncertainties in extrapolating these results to humans, as the main effects are related to nasal epithelium toxicity at the Point of Contact/Portal of Entry (POE), and refined models (e.g., CFD models) are available. POE effects have reduced toxicokinetic uncertainties, CFD models greatly reduce uncertainties in

extrapolation of dose-response results from rats to humans, and there are no know polymorphisms in the one carbon pathway or in formaldehyde dehydrogenases or other less specific aldehyde dehydrogenases that could introduce substantial uncertainties related to intra-species variability.

Additionally, formaldehyde is not metabolized before binding to DNA and proteins. As such, for formaldehyde, uncertainties related to toxicokinetics are greater than toxicodynamic considerations. In contrast, vinyl acetate requires metabolism to acetic acid and acetaldehyde, with acetaldehyde binding to DNA and proteins. As such, characterizing the dose response for acetaldehyde-related DNA adducts addresses both toxicokinetic and toxicodynamic influences that occur after inhalation and before the proximal carcinogen binds to the potential molecular target. As such, there is a greater reduction in toxicodynamic uncertainties for vinyl acetate when compared to formaldehyde.

G. Allow the calculation of risk (probability of response for the endpoint of interest) in the exposed human population?

SILMS methods, as discussed for inhalation exposures, do not afford a direct calculation of risk, unless *de minimus* (essentially zero) risk due to relatively very low (e.g., $1/100^{th}$ or $1/1000^{th}$ of a variable background) or no exposure to the molecular targets is demonstrated. This is because SILMS may only provide molecular dosimetry data for DNA and/or only address one Key Event (e.g., DNA-protein x-links that could affect cell division) or are indicators of an early Key Event such as base-pair adduction rather than mutation. Also, additional key events lie downstream in the cancer MOA. Thus, all Key Events must be considered in the hazard identification and dose-response assessment in order to estimate risk. The biological effect of protein adducts on cell replication rate and the rate of conversion of DNA adducts to mutations both have considerable uncertainties. As such, the results are currently more relevant to determining relative risk and/or ruling out various MOAs than providing point estimates of risk for a continuous risk response. Regarding relative risk, the results are especially relevant to quantitating the added risk of developing nasal tumors when normal endogenous levels of formaldehyde are perturbed and ruling out added risk when normal endogenous levels of formaldehyde are unaffected by inhalation of ppb levels of formaldehyde in air, such as modeled by Starr and Swenberg (2013, 2016).

A BBDR model describing potential human respiratory tract cancer risk from inhaled formaldehyde was described by Conolly et al. (2003; 2004). However, that work predated the availability of adduct data obtained using SILMS. An effort is now underway to update the BBDR modeling to incorporate the newer SILMS adduct data, including the apparent dosimetry threshold at 0.3 ppm. Lastly, the formaldehyde data have been used to rule out systemic effects, such as leukemia (Gentry et al., 2021), since molecular dosimetry conclusively demonstrates that, at concentrations ≤15 ppm, formaldehyde is not distributed beyond the nasal epithelium.

H. Work practically? If the method still requires development, how close is it to practical implementation?

Advancements in SILMS technologies have achieved the sensitivity and specificity to allow both formaldehyde and acetaldehyde DNA adducts to be quantitated at 3-4 orders of magnitude below naturally occurring endogenous adduct levels. As such, for this limited set of chemicals, the technology is ready for practical implementation for at least these chemicals. The majority of this work has been published withing the last five years, with the vinyl acetate data published within the last year. Incorporation of these data into regulatory assessments is limited to the European REACH evaluation of formaldehyde (ECHA, 2019; 2020) where the data were used in a practical manner to support a carcinogenic threshold that lies above the European occupational exposure limit (OEL of 0.3 ppm). As stated by ECHA (2019), before a more recent study (Leng et al., 2019) investigated and identified no detection of DNA adducts at ≤0.3 ppm:

> "SCOEL considers that tumour induction in the nasal mucosa of rats and mice is the result of chronic proliferative processes caused by the cytotoxic effects of the substance in combination with DNA alterations by endogenous and exogenous formaldehyde. At the lowest concentrations investigated so far (0.7 ppm; 0.86 mg/m^3), DNA adducts in the nasal mucosa were still detected. However, DNA adduct levels in the nasal mucosa caused by endogenous, physiological formaldehyde by far exceeded the amounts caused by exogenous formaldehyde (0.7 ppm; 0.86 mg/m^3)."

It is anticipated that SILMS technologies will be used to evaluate the molecular dosimetry for chemicals without endogenous exposures, although the general method does not need further development, considerable chemical-specific SILMS methods will have to be developed to detect and quantify other chemical-specific DNA and protein adducts.

References
(Bold font identifies background documents suggested for review by committee)

ACS. Risk Factors for Nasal and Paranasal Sinus Cancers. American Cancer Society. Webpage accessed Jan 17, 2022.. https://www.cancer.org/cancer/nasal-cavity-and-paranasal-sinus-cancer/causes-risks-prevention/risk-factors.html

Campbell, J.L., Gentry, P.R., Clewell, H.J., and M. Andersen. A Kinetic Analysis of DNA-Deoxy Guanine Adducts in the Nasal Epithelium Produced by Inhaled Formaldehyde in Rats – Assessing Contributions to Adduct Production From Both Endogenous and Exogenous Sources of Formaldehyde. Toxicol. Sci. 177(2), 2020, 325–333. doi: 10.1093/toxsci/kfaa122

Chand, E.RT., Ye, W., Zeng, Y.X. and H.O. Adami. 2021. The Evolving Epidemiology of Nasopharyngeal Carcinoma. Cancer Epidemi, Biomarkers & Prevention. April 13, 2021. https://cebp.aacrjournals.org/content/cebp/30/6/1035.full.pdf

Conolly, R.B., Lilly, P.D., Kimbell, J.S. (2000). Simulation modeling of tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in Fischer 344 rat, Rhesus monkeys, and humans. Env. Health Perspect. 108, Suppl. 5, 919-924, doi: 10.1289/ehp.00108s5919.

Conolly, R.B., Kimbell, J.S., Janszen, D., Schlosser, P.M., Kalisak, D., Preston, J., and Miller, F.J. (2003). Biologically motivated computational modeling of formaldehyde carcinogenicity in the F344 rat. Toxicol. Sci. 75, 432 – 447.

Conolly, R.B., Kimbell, J.S., Janszen, D., Schlosser, P.M., Kalisak, D., Preston, J., and Miller, F.J. (2004). Human respiratory tract cancer risks of inhaled formaldehyde: Dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol. Sci. 82, 29 – 296.

Conolly, R.B., Campbell, J.L., Clewell, H.J., Schroeter, J., Kimbell, J.S. and P. R. Gentry. 2022. Relative Contributions of Endogenous and Exogenous Formaldehyde to Formation of Deoyguanosine and DNA-Protein Crosslink Adducts in Rat Nasal Mucosa. In Preparation

ECHA. 2019. Worker exposure to formaldehyde and formaldehyde releasers. European Chemicals Agency. Version Number: 1. 12 July 2019.

ECHA. 2020. Committee for Risk Assessment (RAC), Committee for Socio-economic Analysis (SEAC) Background Document to the Opinion on Annex XV dossier proposing restriction on formaldehyde and formaldehyde releasers. ECHA/RAC/RES-O-0000006740-76-01/F ECHA/SEAC/RES-O-0000006931-71-01/F. Final. September 17, 2020.

Hsiao, Y-C., Liu, C-W, Hoffman, G., Fang, C. and K. Lu. 2021. Molecular Dosimetry of DNA Adducts in rats Exposed to Vinyl Acetate Monomer. Toxicol Sci. https://academic.oup.com/toxsci/advance-article/doi/10.1093/toxsci/kfab140/6460835

Gentry PR, Thompson C, Franzen A, Salley J, Albertini R, Lu K, Greene T. 2021. Using mechanistic information to support evidence integration and synthesis: A Case Study with Formaldehyde and Leukemia. Critical Reviews in Toxicology. 50(10):885-918 https://doi.org/10.1080/10408444.2020.1854678

Lai Y, Yu R, Hartwell HJ, Moeller BC, Bodnar WM, Swenberg JA. 2016. Measurement of endogenous versus exogenous formaldehyde-induced DNA-protein crosslinks in animal tissues by stable isotope labeling and ultrasensitive mass spectrometry. Cancer Research, 76(9): 2652-2661.

Leng, J., Liu, C-W., Hartwell H.J., Yu R., Lai, Y., Bodnar W.M., Lu, K., and J.A. Swenberg. 2019. Evaluation of inhaled low-dose formaldehyde-induced DNA adducts and DNA-protein cross-links by liquid chromatography-tandem mass spectrometry. Archives of Toxicology, 93(3): 763-773.

Liu, C-W., Hsiao, Y-C., Hoffman, G. and K. Lu. 2021. LC-MS/MS Analysis of the Formation and Loss of DNA Adducts in Rats Exposed to Vinyl Acetate Monomer Through Inhalation. Chem. Res. Toxicol. 34(3): 793–803. https://dx.doi.org/10.1021/acs.chemrestox.0c00404

Lu, K., Moeller, B.C. and J.A. Swenberg. 2011. Further Considerations for the Implausibility of Leukemia Induction by Formaldehyde. Letter to the Editor. Toxicological Sciences. 120(1): 223.

Lu, K., Hsiao, Y-C, Liu., C-W., Schoeny, R., Gentry, R. and T. Starr. 2021. A Review of Stable Isotope Labeling and Mass Spectrometry Methods to Distinguish Exogenous from Exogenous DNA Adducts and Improve Dose-Response Assessments. Chem Res Toxicol. https://pubs.acs.org/doi/10.1021/acs.chemrestox.1c00212

McGregor, D., Bolt, H., Cogliano, V. Richter-Reichhelm, H.B. 2006. Formaldehyde and glutaraldehyde and nasal cytotoxicity: case study within the context of 2006 IPCS Human Framework for the Analysis of a Cancer Mode of Action for Humans. Crit Rev. Toxicol. 36(10):921-835.

Starr, T.B. and J.A. Swenberg. 2016. The bottom-up approach to bounding potential low-dose cancer risks from formaldehyde: An update. Reg Tox and Pharm. 77:167-174

Swenberg J, Lu K, Moeller B, Gao L, Upton P, Nakamura J, Starr JB. 2011. Endogenous versus exogenous DNA adducts: their role in carcinogenesis, epidemiology and risk assessment. Toxicological Sciences, 120: 130-145.

Thompson, C.M., Gentry, R., Fitch, S., Lu, K. & H.J. Clewell. 2020. An updated mode of action and human relevance framework evaluation for Formaldehyde-Related nasal tumors, Critical Reviews in Toxicology, 50:10, 919-952, DOI:10.1080/10408444.2020.1854679

Yu R, Lai Y, Hartwell HJ, Moeller BC, Doyle-Eisele M, Kracko D, Bodnar WM, Starr TB, Swenberg JA. 2015. Formation, accumulation, and hydrolysis of endogenous and exogenous formaldehyde-induced DNA damage. Toxicological Sciences, 146(1): 170-182.

Yun-C Hsiao, Y-C., Liu, C-W., Hoffman, G., Fang, C. and K. Lu. 2021. Molecular Dosimetry of DNA Adducts in Rats Exposed to Vinyl Acetate Monomer. Toxicol. Sci. 1-11. **https://pubmed.ncbi.nlm.nih.gov/34904679/**