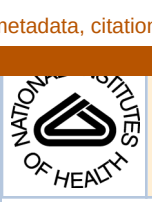

Published in final edited form as:

Chem Res Toxicol. 2011 February 18; 24(2): 162–164. doi:10.1021/tx1004166.

Determination of N^2-hydroxymethyl-dG Adducts in Nasal Epithelium and Bone Marrow of Non-human Primates following $^{13}CD_2$-Formaldehyde Inhalation Exposure

Benjamin C. Moeller[†], **Kun Lu**[‡], **Melanie Doyle-Eisele**[‖], **Jacob McDonald**[‖], **Andrew Gigliotti**[‖], and **James A. Swenberg**[†,‡,*]

[†]Curriculum in Toxicology, University of North Carolina – Chapel Hill

[‡]Department of Environmental Sciences and Engineering, University of North Carolina – Chapel Hill

[‖]Lovelace Respiratory Research Institute, Albuquerque, NM

Abstract

The presence of endogenous and exogenous N^2-hydroxymethyl-dG adducts in DNA from nasal mucosa and bone marrow of cynomolgus macaques exposed to 1.9 and 6.1 ppm of [$^{13}CD_2$]-formaldehyde for 6 hours a day for 2 consecutive days was investigated using a highly sensitive nano-UPLC-MS/MS method with a Limit of Detection of 20 amol. Both exogenous and endogenous adducts were readily detected and quantified in the nasal tissues of both exposure groups, with an exposure dependent increase in exogenous adducts observed. In contrast, only endogenous adducts were detectable in the bone marrow, even though ~10 times more DNA was analyzed.

Formaldehyde is a ubiquitous chemical that is used in a large number of industrial activities and found in many consumer products. In addition to occupational and environmental exposures, formaldehyde is endogenously produced as part of the one carbon pool and is present in the blood at about 0.1 mM (1). IARC has classified formaldehyde as a carcinogen, causing squamous cell carcinoma in nasal tissue of rats, and nasopharyngeal cancer in humans. There is also limited evidence from epidemiological studies for an association with myeloid leukemia (1–3). The US Environmental Protection Agency has recently released its external review draft *Toxicological Review of Formaldehyde-Inhalation Assessment*, which is currently under expert review by the National Academy of Sciences. Detailed studies of formaldehyde derived DNA damage are needed to better understand the extent of endogenous and exogenous damage in non-human primates.

Initial investigations into the carcinogenicity of formaldehyde found dramatic increases in the incidence of squamous cell carcinomas following long-term exposure to rats at concentrations higher than 6 ppm (4–6). Additional studies in rodents and primates have investigated formaldehyde damage, finding non-linear increases in markers of toxicity and DNA damage (1;7). Studies in rats have provided extensive information regarding formaldehyde derived DNA damage; however, there are significant differences in the

[*]To whom correspondence should be addressed: Department of Environmental Sciences and Engineering Gillings School of Public Health, Campus Box, 7431, The University of North Carolina, Chapel Hill, NC 27599-7431. Telephone: (919)966-6123. jswenber@email.unc.edu.

Supporting Information available: Experimental details and method validation data are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

structure of nasal passages and disposition of inhaled chemicals between rodents and primates, including humans (8). These differences and oral/nasal breathing patterns make primates a more appropriate model of human exposure. Several models have been developed to simulate formaldehyde derived damage in rodents, primates and humans (1;9;10).

Formaldehyde is highly reactive and can covalently bind to both proteins and DNA, forming formaldehyde-DNA monoadducts, DNA-formaldehyde cross-links and DNA-protein cross-links (1;11). The endogenous presence of formaldehyde and the high concentrations normally found in cells result in the endogenous formation of identical DNA adducts, making quantitation of exogenous and endogenous damage problematic. The majority of previous research focused on the detection of DNA-protein cross-links, which primarily relied on physical separation and detection methodologies that either could not adequately differentiate between endogenous and exogenous formaldehyde damage, or could only detect exogenous damage. More recent studies by Lu et al, have utilized a combination of isotope labeled [$^{13}CD_2$]-formaldehyde exposures to rats and quantitation of DNA adducts including N^2-hydroxymethyl-dG by mass spectrometry. This allowed unambiguous determinations of exogenous and endogenous formaldehyde derived DNA damage (11). Our laboratory has recently expanded upon these studies, conducting experiments measuring N^2-hydroxymethyl-dG using a newly developed more sensitive nano-UPLC-MS/MS method to determine the molecular dosimetry of hydroxymethyl-dG adducts in rats (12). Here, we present the first data using this more sensitive and selective approach for the detection of exogenous and endogenous formaldehyde induced DNA damage in non-human primates.

Two groups (n = 4 animals per group) of cynomolgus macaques (*Macaca fascicularis*) were whole body exposed to either 1.9 or 6.1 ppm [$^{13}CD_2$]-formaldehyde for 2 consecutive days (6 hours per day). At the end of exposure on the second day, the primates were euthanized and necropsy performed within 3 hours. The nasal mucosa from maxilloturbinates and bone marrow were collected, flash frozen in liquid nitrogen and stored at −80°C until analysis. DNA was extracted, reduced with $NaCNBH_3$ to convert N^2-hydroxymethyl-dG adducts to more stable N^2-methyl-dG adducts. Subsequently, 20 fmol of the internal standard ($^{15}N_5{}^{13}C_{10}$-methyl-dG) was added, DNA digested with DNase I, alkaline phosphatase and phosphodiestersase, followed by the separation of N^2-methyl-dG by HPLC and drying of fractions under vacuum. Both endogenous and exogenous N^2-methyl-dG were detected and quantitated using a sensitive nano-UPLC-MS/MS method employing select reaction monitoring with a limit of detection of 20 amol on column (Figure S1). In bone marrow and nasal tissue, the presence of both endogenous (m/z 282.2 → 166.1) and exogenous (m/z 285.2 → 169.1) N^2-hydroxymethyl-dG adducts, along with the internal standard (m/z 297.2 → 176.1) were monitored, as shown in figure 1.

The endogenous and exogenous N^2-hydroxymethyl-dG levels found in both tissues are summarized in Table 1. In the nasal maxilloturbinate DNA, exogenous N^2-hydroxymethyl-dG was present at 0.26 ± 0.04 and 0.41 ± 0.05 adducts/10^7 dG following the 1.9 and 6.1 ppm exposures, respectively. Endogenous N^2-hydroxymethyl-dG adducts were present in the nasal DNA of all animals studied, with an average of 2.24 ± 0.50 adducts/10^7 dG.

In bone marrow, no exogenous adducts were detected, even though ~10-fold higher amounts of DNA were analyzed. Endogenous N^2-hydroxymethyl-dG adducts were present at 17.5 ± 2.6 and 12.4 ± 3.6 adducts/107 dG in bone marrow DNA from the 1.9 and 6.1 ppm exposures, respectively. The lack of exogenous adducts with the corresponding presence of endogenous adducts is clearly shown in Figure 1. In contrast to the nasal tissue, much higher numbers of endogenous N^2-hydroxymethyl-dG adducts were present in bone marrow. It is unclear why the numbers of endogenous adducts in primates are high, but preliminary evaluations of other tissues were also much higher than was previously found in rats.

In a recent study by Lu et al (12), N^2-hydroxymethyl-dG exogenous and endogenous adducts were quantified in rats using the same methodology described in this paper following a single 6 hour exposure to labeled formaldehyde. The number of exogenous adducts quantified after a 1 day exposure to rats and a 2 day exposure to monkeys were similar following 1.9 ppm $^{13}CD_2O$ exposure, but were ~2.5 fold lower in primates exposed to 6.1 ppm. This decrease in exogenous adducts, compared to the rat, may be partially explained by the differences in nasal anatomy and breathing patterns between the two species (8). Rats, unlike humans and monkeys are obligate nose breathers, which may explain the higher amounts of exogenous adducts in rat nasal tissue. Additional tissue sites from the nasal and respiratory tract, as well as numerous sites distant to the point of contact were collected at necropsy and will be analyzed for a more comprehensive report on this study to better characterize the distribution of exogenous and endogenous adducts in non-human primates following formaldehyde inhalation exposure.

Our new nano-UPLC-MS/MS method permits one to calculate the maximum number of exogenous formaldehyde adducts that could have been missed in a sample of bone marrow. For example, in a 312 μg DNA sample, less than 1 exogenous N^2-HO$^{13}CD_2$-dG could be present in a sample that had 13,900 identical endogenous formaldehyde adducts. This provides a unique perspective on the plausibility that inhaled formaldehyde gets to bone marrow and is causally associated with the induction of myeloid leukemia. How could that one exogenous adduct have a biological effect, but the other 13,900 do not? Such data demonstrate the utility of conducting stable isotope exposures when a chemical induces DNA adducts that are identical to endogenous adducts. It also strengthens the arguments supporting the need to consider the biology associated with high numbers of endogenous DNA adducts when extrapolating to risks associated with very low exposures to chemicals.

These data clearly show the formation of exogenous formaldehyde adducts in nasal DNA of primates and the lack of formation of exogenous DNA adducts in the bone marrow. Lower amounts of exogenous adducts were found in the nasal tissues of primates exposed to formaldehyde for 2 days, compared to similar studies conducted in rats exposed for one day. Furthermore, primates had much higher endogenous adducts. Primates will have a lower ratio of exogenous/endogenous adduct arising from inhalation exposures, suggesting that risks from inhaled formaldehyde exposures may also be lower. Additional studies are underway on the expanded list of tissues to better characterize the potential for exogenous DNA damage caused by inhalation exposure to formaldehyde in the respiratory tract and distant tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Support. This work was supported in part by NIH grants P30-ES10126, P42-ES05948 and T32-07126, and a grant from the Formaldehyde Council Inc to J.A.S.

Reference List

1. IARC. Formaldehyde: 2-Butoxyethanol and 1-*tert*-Butoxypropan-2-ol. IARC Monogr. Eval. Carcinog. Risks Hum. 2006; 88:1–287. [PubMed: 17366697]
2. Beane Freeman LE, Blair A, Lubin JH, Stewart PA, Hayes RB, Hoover RN, Hauptmann M. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute cohort. J. Natl. Cancer Inst. 2009; 101:751–761. [PubMed: 19436030]

3. Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbard AE, Hua M, Blair A, Galvan N, Ruan X, Alter BP, Xin KX, Li S, Moore LE, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaport SM, Huang H, Fraumeni JF Jr, Smith MT, Lan Q. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol. Biomarkers Prev. 2010; 19:80–88. [PubMed: 20056626]

4. Swenberg JA, Kerns WD, Mitchell RI, Gralla EJ, Pavkov KL. Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. Cancer Res. 1980; 40:3398–3402. [PubMed: 7427950]

5. Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res. 1983; 43:4382–4392. [PubMed: 6871871]

6. Monticello TM, Swenberg JA, Gross EA, Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE, Morgan KT. Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res. 1996; 56:1012–1022. [PubMed: 8640755]

7. Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck Hd. Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fundam. Appl. Toxicol. 1991; 17:409–428. [PubMed: 1765228]

8. Harkema JR, Carey SA, Wagner JG. The nose revisted: A brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol. Path. 2006; 34:252–269. [PubMed: 16698724]

9. Conolly RB, Lilly PD, Kimbell JS. Simulation modeling of the tissue disposition of formaldehyde to predict nasal DNA-protein cross-links in Fischer 344 rats, rhesus monkeys, and humans. Environ. Health Perspect. 2000; 108 Suppl 5:919–924. [PubMed: 11036001]

10. Morgan KT, Kimbell JS, Monticello TM, Patra AL, Fleishman A. Studies of inspiratory airflow patterns in the nasal passages of the F344 rat and rhesus monkey using nasal molds: relevance to formaldehyde toxicity. Toxicol. Appl. Pharmacol. 1991; 110:223–240. [PubMed: 1891770]

11. Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA. Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol. Sci. 2010; 116:441–451. [PubMed: 20176625]

12. Lu K, Moeller BC, Doyle-Eisele M, McDonald J, Swenberg JA. Molecular dosimetry of N^2-hydroxymethyl-dG DNA adducts in rats exposed to formaldehyde. Chem Res Toxicol. 2010 (In Press).

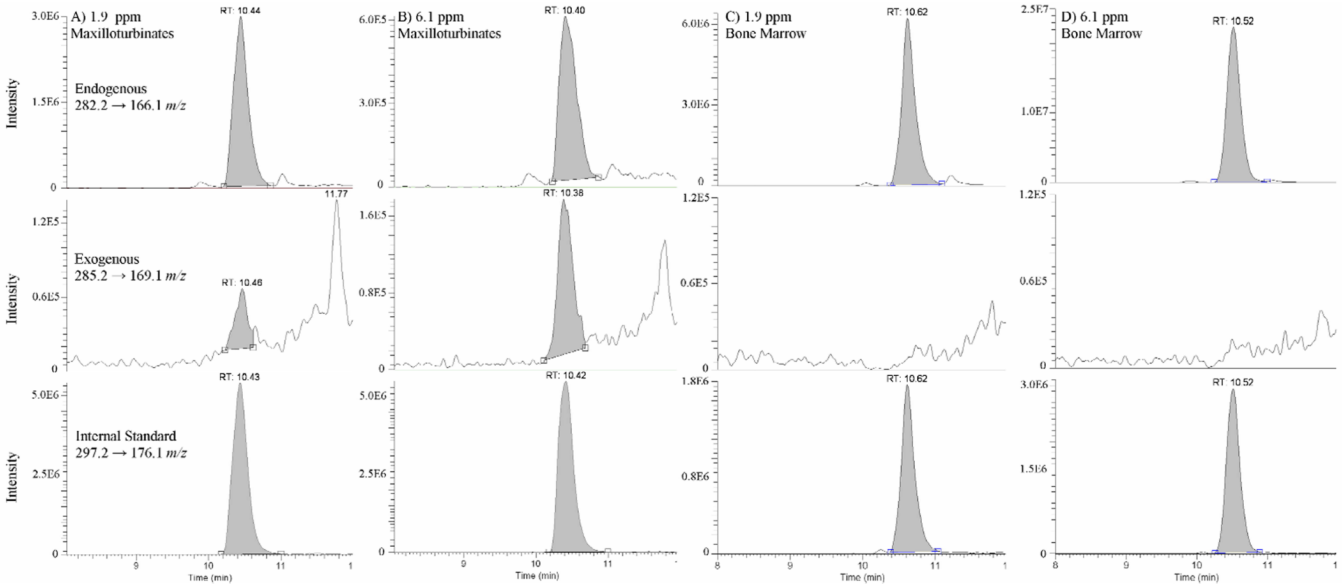

Figure 1.

Nano-UPLC-MS/MS Chromatograms. Chromatograms showing exogenous and endogenous N^2-hydroxymethyl-dG adduct formation in primate nasal maxilloturbinates (A and B) following 2 days of 6 hour exposures at 1.9 and 6.1 ppm [$^{13}CD_2$]-formaldehyde, respectively. In contrast, only endogenous adducts were present in bone marrow (C and D).

Table 1

Endogenous and Exogenous N^2-hydroxymethyl-dG levels.

Tissue	Exposure Concentration (ppm)	Exogenous Adducts/10^7 dG	Endogenous Adducts/10^7 dG
Nasal	1.9	0.26 ± 0.04	2.50 ± 0.40*
Maxilloturbinate	6.1	0.41 ± 0.05	2.05 ± 0.54
Bone Marrow	1.9	n.d.	17.5 ± 2.6
	6.1	n.d.	12.4 ± 3.6

n.d. = not detected,

* n = 3 samples