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Molecular Dosimetry of N^2-hydroxymethyl-dG DNA Adducts in Rats Exposed to Formaldehyde

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Abstract

In this study, both endogenous and exogenous N^2-hydroxymethyl-dG adducts in nasal DNA of rats exposed to 0.7, 2, 5.8, 9.1 or 15.2 ppm [$^{13}CD_2$] formaldehyde for 6 h were quantified by a highly sensitive nano-UPLC-MS/MS method. Our data clearly demonstrated that exogenous formaldehyde DNA adducts form in a highly nonlinear fashion, with a 21.7-fold increase in exposure causing a 286-fold increase in exogenous adducts. The ratio of exogenous/endogenous DNA adducts demonstrated that endogenous DNA adducts dominated at low exposures, comprising more than 99%. In contrast, exogenous adducts were not detectable in bone marrow of rats exposed to 15.2 ppm [$^{13}CD_2$] formaldehyde.

Formaldehyde, one of the top 20 high volume production industrial chemicals, is used in a wide spectrum of applications. Therefore, formaldehyde exposure from occupational and environmental sources is very common. It was estimated that more than 2 million workers and professionals in the United States are exposed to formaldehyde. Formaldehyde is classified as a known human and animal carcinogen according to IARC (1) causing nasopharyngeal cancer in humans and squamous cell carcinomas in the nasal passages of rats (2;3). In addition, epidemiological studies provided limited evidence for the induction of leukemia in human, but the results are inconsistent across different studies (4-6) and no mechanisms for the induction of leukemia have been established (4). Formaldehyde's well known toxicity and carcinogenicity, coupled with wide spread human exposure has raised long-standing public concerns over its safety. Recently, formaldehyde concentrations in FEMA trailers used for emergency housing after Hurricane Katrina brought additional awareness and debates on safe levels of formaldehyde exposure. Several international and national regulatory agencies have updated their risk assessment documents on formaldehyde since last year. The US Environmental Protection Agency just released its external review draft Toxicological Review of Formaldehyde-Inhalation Assessment on 2 June 2010 and it is currently under expert review by the National Academy of Sciences.

Formaldehyde is a chemical that has been extensively studied over the last 30 years (3;7). Numerous studies have demonstrated that both genotoxicity and cytotoxicity contribute to

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Supporting Information available: Experimental method details, scheme of analytical approach and typical chromatograms and calibration curve. This material is available free of charge via the Internet at http://pubs.acs.org.

the carcinogenic mode of action of formaldehyde in nasal tissues (1). The assessment of formaldehyde risk is complex due to the fact that both endogenous and exogenous sources of formaldehyde are present in the body and the mode of action is complex. Exogenous formaldehyde enters the body through inhaled environmental exposure such as vehicle emissions, building materials, and tobacco smoke, as well as through metabolism of foods, chemicals and drugs. However, endogenous formaldehyde is also present as an essential metabolic intermediate in all living cells. How can we accurately assess the risk of exogenous formaldehyde in the presence of a substantial background of endogenous formaldehyde? It should be pointed out that any measurements of biomarkers will be a mixture of the contribution of endogenous and exogenous formaldehyde, unless specific approaches capable of unequivocally differentiating between them are used.

Cancer is a disease of mutations and the formation of DNA adducts is a key event of both mutagenesis and carcinogenesis. Complicated interplay between reactivity of a chemical, distribution and metabolism, adduct stability, DNA repair, cell death and proliferation determine the amount, tissue distribution and molecular dose over time for DNA adducts. Therefore, DNA adducts have been widely used as a molecular dosimeter to better reflect the internal dose of a genotoxic chemical in target tissues following exposure. Previously, DNA-protein cross-link (DPC)[1] data measured by either physical chemistry or enzyme digestion approaches have been applied as a surrogate to assess the risk of formaldehyde exposure (1). However, these DPC data do not represent a formaldehyde-specific biomarker that differentiates between endogenous and exogenous sources of formaldehyde. Formaldehyde induced endogenous DPC are always present, but no quantitative data are available. Furthermore, available data on stability and repair are not quantitative, as the methods do not differentiate between DPC and cleavage to short peptides that lack the needed physical chemistry of the methods used.

In our initial study, we demonstrated that inhaled [$^{13}CD_2$]-formaldehyde induced [$^{13}CD_2$]-N^2-hydroxymethyl-dG adducts in nasal epithelium of rats exposed to 10 ppm [$^{13}CD_2$]-formaldehyde, but not in any tissues distant to the site of contact examined, even though ~ 5 times more DNA was analyzed for distant tissues (7). In this study, we advanced our investigations by exposing rats to 0.7, 2, 5.8, 9.1, or 15.2 ppm [$^{13}CD_2$]-formaldehyde for 6 h, which modeled the exposures used previously in a 2-year carcinogenicity bioassay (2). Moreover, an improved nano-UPLC-ESI-MS/MS-SRM method was developed to detect formaldehyde DNA adducts, which was 10-fold more sensitive than our previously reported capillary-LC-ESI-MS/MS-SRM method (7). Using this unique approach, both formaldehyde endogenous and exogenous DNA adducts were quantified simultaneously in nasal epithelial DNA of rats exposed to [$^{13}CD_2$]-formaldehyde covering a 21.7-fold difference in concentration.

The outline of our analytical approach for formaldehyde-induced N^2-hydroxymethyl-dG adducts is illustrated in Figure S1. Briefly, rats were exposed to [$^{13}CD_2$]-formaldehyde for 6 h, sacrificed within 2 h following exposure. DNA was isolated from nasal respiratory epithelium, followed by incubation with $NaCNBH_3$ for 6 h to convert N^2-hydroxymethyl-dG to stable N^2-methyl-dG. After enzymatic digestion, the fractions containing N^2-methyl-dG adducts and corresponding internal standard were collected by HPLC. After drying by speed vacuum, adducts were analyzed by a highly sensitive nano-UPLC-MS/MS-SRM method with 20 amol limit of detection on the column. Figure S2 gives a typical chromatogram and calibration curve used for the quantitation of DNA adducts.

[1]Abbreviations: DPC, DNA-protein crosslinks.

Figure 1 shows the typical nano-UPLC-MS/MS-SRM chromatograms of N^2-methyl-dG adducts in nasal DNA of rats exposed to 0.7 and 15 ppm [$^{13}CD_2$]-formaldehyde (Figure S3 gives more typical chromatograms for other exposures). The peak corresponding to the specific transition of m/z 282.2→m/z 166.1 and the same retention time with [$^{13}C_{10}{}^{15}N_5$]-N^2-methyl-dG internal standard unambiguously identified endogenous formaldehyde-induced N^2-hydroxymethyl-dG, as shown by the peak in the top panel of Figure 1. The signal corresponding to the transition of m/z 285.2→m/z 169.1 coeluted with the internal standard and is attributed to [$^{13}CD_2$]-N^2-hydroxymethyl-dG arising from exogenous [$^{13}CD_2$]-formaldehyde. As shown in Figure 1, the signals of endogenous DNA adduct peaks were similar in nasal DNA of rats exposed to different concentrations of formaldehyde for 6 h. In contrast, increased [$^{13}CD_2$]-formaldehyde exposures induced significantly higher exogenous DNA adducts in nasal epithelium of rats.

Table 1 lists the quantitative results for both endogenous and exogenous formaldehyde-DNA adducts. The number of exogenous N^2-hydroxymethyl-dG adducts induced was 0.039±0.019, 0.19±0.08, 1.04±0.24, 2.03±0.43 and 11.15±3.01 adducts/10^7 dG for 0.7, 2.0, 5.8, 9.1 and 15.2 ppm [$^{13}CD_2$]-formaldehyde exposure for 6 hours, respectively. Thus, the exogenous adducts were formed in a highly nonlinear fashion, as demonstrated by the fact that a 21.7- fold increase in exposure (0.7 to 15.2 ppm) formed 286-fold higher amounts of exogenous DNA adducts in rat nasal epithelium. This effect occurred as a continuum, as shown by examining the number of [$^{13}CD_2$]-adducts induced per ppm of exposure. Here, 0.06 adducts/10^7 dG were formed per ppm at 0.7 ppm, 0.10 adducts/10^7 dG at 2.0 ppm, 0.18 adducts/10^7 dG at 5.8 ppm, 0.22 adducts/10^7 dG at 9.1 ppm, and 0.73 adducts/10^7 dG at 15.2 ppm [$^{13}CD_2$]-formaldehyde. Thus, the number of [$^{13}CD_2$]-N^2-hydroxymethyl-dG adducts increased more than 12-fold per ppm between the lowest and highest exposures.

In contrast, the amount of endogenous N^2-hydroxymethyl-dG did not exhibit a concentration dependent effect, as 3.62±1.33, 6.09±3.03, 5.51±1.06, 3.41±0.46 and 4.24±0.92 adducts/10^7 dG were present at 0.7, 2.0, 5.8, 9.1 and 15.2 ppm [$^{13}CD_2$]-formaldehyde exposures, respectively. These results demonstrate that a 6h [$^{13}CD_2$]-formaldehyde exposure did not change the number of endogenous dG adducts in nasal epithelial DNA. The amount of endogenous N^2-hydroxymethyl-dG in nasal DNA of rats, calculated for all rats combined was 4.7±1.8 adducts/10^7 dG.

Since the endogenous N^2-hydroxymethyl-dG did not change in an exposure related manner during the 6 hour formaldehyde exposure, exogenous DNA adducts were normalized by the corresponding endogenous adduct number for each animal to minimize individual variability, which was calculated as the ratio of exogenous versus endogenous adducts. As shown in Figure 2, the ratio was 0.011±0.001, 0.033±0.006, 0.19±0.04, 0.60±0.17 and 2.79±1.08 for 0.7, 2.0, 5.8, 9.1 and 15.2 ppm formaldehyde exposure, respectively. Examined another way, if the number of exogenous adducts formed by a single 6 hour exposure to 0.7 ppm [$^{13}CD_2$]-formaldehyde (0.039 ± 0.19 adducts/10^7 dG) is compared with the overall average number of endogenous formaldehyde adducts (4.7±1.8 adducts/10^7 dG), that means that only 83 out of 10,000 formaldehyde adducts arise from the 0.7 ppm exposure for 6 hours. Placing this in a more general perspective, if a rat was housed for 6 hours in a FEMA trailer that had the median concentration of formaldehyde reported by the CDC (77 ppb) (http://www.cdc.gov/nceh/ehhe/trailerstudy/pdfs/FEMAFinalReport.pdf), the exposure would contribute only 91 out of 100,000 adducts. Likewise, the US Environmental Protection Agency draft risk assessment for formaldehyde sets 0.07 ppb as the safe level of formaldehyde. A 6 hr exposure to 0.07 ppb exposure, would induce 83 exogenous DNA adducts out of 100,000,000 formaldehyde adducts.

Bone marrow from the rats exposed to 15.2 ppm [$^{13}CD_2$]-formaldehyde was also analyzed with our more sensitive nano-UPLC-MS/MS method and exogenous formaldehyde adducts were below the detection limit of 20 amol. In contrast, endogenous N^2-hydroxymethyl dG adducts were ~15 adducts/10^7 dG. Thus, less than 1 [$^{13}CD_2$]-adduct could be present in 1,500 identical endogenous adducts in a rat exposed to 15.2 ppm [$^{13}CD_2$]-formaldehyde for 6 hours. It is highly implausible that this one adduct could induce malignant transformation in the bone marrow when 1500 endogenous adducts do not.

In conclusion, this study generated the first molecular dosimetry data using formaldehyde-specific DNA biomarkers. Highly sensitive mass spectrometry coupled with the use of isotope labeled formaldehyde allowed us to differentiate and quantify DNA adducts arising from both endogenous and exogenous formaldehyde. The contribution of exposure under a substantial endogenous background of formaldehyde was unambiguously measured. We have demonstrated that formaldehyde induces exogenous DNA adducts in a highly nonlinear fashion. Examination of the ratio of exogenous versus endogenous formaldehyde DNA adducts clearly demonstrates that endogenous DNA adducts predominate at low ppm doses and that ppb exposures contribute miniscule amounts of exogenous DNA adducts. The data generated in this study provide new scientific evidence for the assessment of risk resulting from formaldehyde exposure through inhalation. Our approach emphasizing the relationship between exogenous and endogenous DNA adducts, is also valuable to assess the risk for other chemicals that form DNA adducts identical to endogenous DNA adducts. Furthermore, since all cells contain significant numbers of endogenous DNA adducts, the role of this ever present background needs to be considered in low dose risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

(1). IARC. Formaldehyde, 2-Butoxyethanol and 1-*tert*-Butoxypropan-2-ol. IARC Monogr. Eval. Carcinog. Risks Hum. 2006; 88:1–287. [PubMed: 17366697]

(2). 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]

(3). 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]

(4). 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]

(5). Freeman, L. E. Beane; 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]

(6). Coggon D, Harris EC, Poole J, Palmer KT. Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J. Natl. Cancer Inst. 2003; 95:1608–1615. [PubMed: 14600093]

(7). 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]

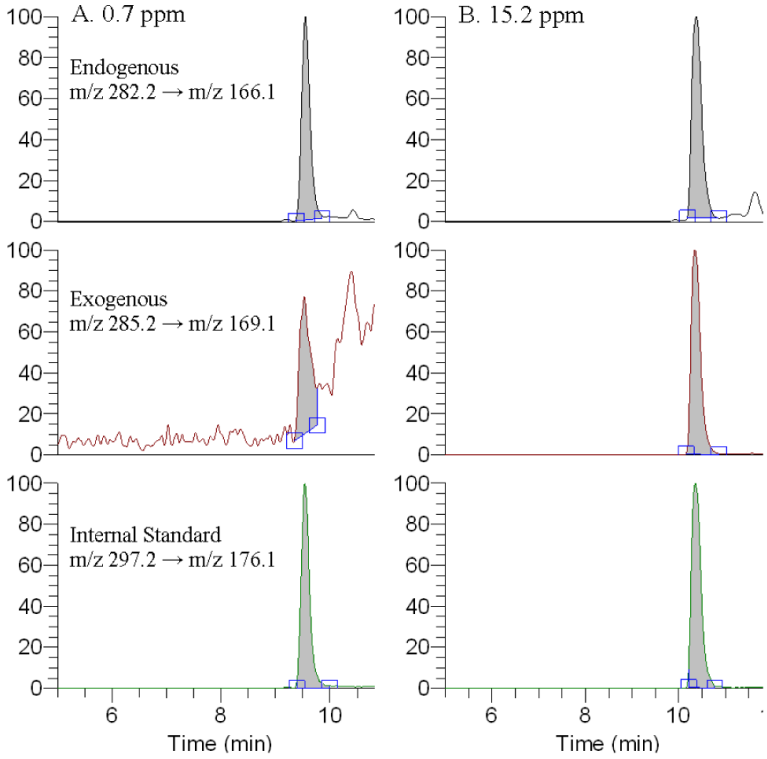

Figure 1.

Typical nano-LC-ESI-MS/MS SEM chromatograms of formaldehyde-induced N^2-me-dG DNA addcts in nasal DNA of rats exposed to 0.7 (A), or 15.2 (B) [$^{13}CD_2$]-formaldehyde for 6 h. Four to six samples were pooled for A; one sample was used for B.

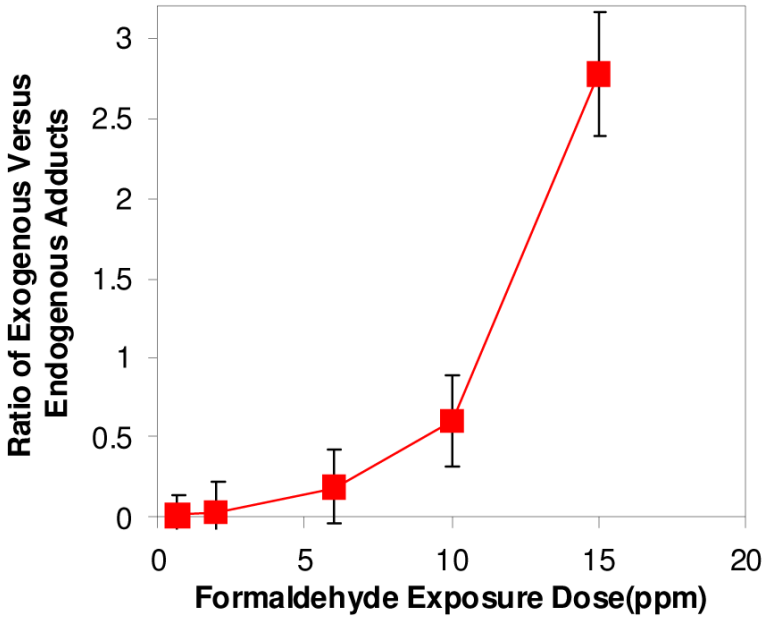

Figure 2.
Exposure-response of ratios of exogenous/endogenous formaldehyde-DNA adducts in nasal epithelium of rats exposed to [$^{13}CD_2$]-formaldehyde for 6 h.

Table 1

Formaldehyde-induced N^2-hydroxymethyl-dG adducts in nasal epithelium of rats exposed to [$^{13}CD_2$]-formaldehyde for 6 h

Exposure (ppm)	Endogenous dG adduct (adducts/10^7 dG)	Exogenous dG adducts (adducts/10^7 dG)
0.7± 0.2	3.62± 1.33*	0.039± 0.019
2.0± 0.1	6.09± 3.03#	0.19± 0.08
5.8± 0.5	5.51± 1.06$	1.04± 0.24
9.1± 2.2	3.41± 0.46	2.03± 0.43
15.2± 2.1	4.24± 0.92	11.15± 3.01

*4-6 rat samples were combined for each mass spectrometry measurement; n=3

#2 rat samples were combined for each mass spectrometry measurement; n=4

$no rat samples were combined for 5.8, 9.1 and 15 ppm groups; typical n=5