



HHS PUBLIC ACCESS

Author manuscript

Chem Res Toxicol. Author manuscript; available in PMC 2018 February 09.

Published in final edited form as:

Chem Res Toxicol. 2017 August 21; 30(8): 1572–1576. doi:10.1021/acs.chemrestox.7b00075.

N⁶-Formyllysine as a biomarker of formaldehyde exposure: Formation and loss of N⁶-formyllysine in nasal epithelium in long-term, low-dose inhalation studies in rats

Bahar Edrissi[†], Koli Taghizadeh[‡], Benjamin C. Moeller^{§,||}, Rui Yu[§], Dean Kracko^{||}, Melanie Doyle-Eisele^{||}, James A. Swenberg^{§,*}, and Peter C. Dedon^{†,‡,*}

[†]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA

[‡]Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA

[§]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, 27514, USA

^{||}Lovelace Respiratory Research Institute, Albuquerque, NM, 87108, USA

Abstract

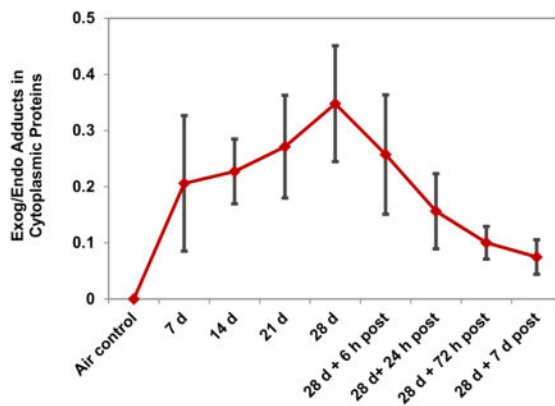
Exposure to both endogenous and exogenous formaldehyde has been established to be carcinogenic, likely by virtue of forming nucleic acid and proteins adducts such as N⁶-formyllysine. To better assess N⁶-formyllysine as a biomarker of formaldehyde exposure, we studied accumulation of N⁶-formyllysine adducts in tissues of rats exposed by inhalation to 2 ppm [¹³C²H₂]-formaldehyde for 7, 14, 21, and 28 days (6h/day) and investigated adduct loss over a 7-day post-exposure period using liquid chromatography-coupled tandem mass spectrometry. Our results showed formation of exogenous adducts in nasal epithelium and to some extent in trachea, but not in distant tissues of lung, bone marrow, or white blood cells, with a 2-fold increase over endogenous N⁶-formyllysine over a 3-week exposure period. Post-exposure analyses indicated a bi-exponential decay of N⁶-formyllysine in proteins extracted from different cellular compartments, with half-lives of ~25h and ~182h for the fast and slow phases, respectively, in cytoplasmic proteins. These results parallel the behavior of DNA adducts and DNA-protein cross-links, with protein adducts cleared faster than DNA-protein cross-links, and point to the potential utility of N⁶-formyllysine protein adducts as biomarkers of formaldehyde.

Graphical Abstract

^{*}Corresponding Authors. Department of Biological Engineering, 56-787B, Massachusetts Institute of Technology, Cambridge, MA 02139; tel: 617-253-8017; pcdedon@mit.edu; Department of Environmental Science and Engineering, UNC, Chapel Hill, NC 27514; tel: 919-966-6139; jswen-ber@email.unc.edu.

Supporting Information

Quantitative exogenous and endogenous N⁶-formyllysine adducts data. This material is available free of charge via the Internet at <http://pubs.acs.org>.



Introduction

Human exposure to the electrophilic, genotoxic carcinogen formaldehyde (FA) is complicated by the presence of both endogenous and exogenous sources. FA is a widespread industrial chemical with a global production of over 20 million tons per year and is present in a variety of consumer products.¹ Moreover, formaldehyde is a naturally occurring cellular metabolite and is produced in humans in a variety of endogenous cellular processes.¹⁻³ Given these environmental and endogenous sources of FA, it is not surprising that humans sustain relatively high FA concentrations in plasma, from ~10 to 100 μM .⁴

FA exposure has long been recognized to have significant health risks due to its toxicity and carcinogenicity.^{1,2,4,5} It is classified as a known human and rodent carcinogen by IARC, resulting in nasopharyngeal cancer in humans and nasal squamous cell carcinomas in rats.¹⁻³ However, the potential role of inhaled FA in causing leukemia is still a matter of debate due to limited evidence from epidemiological studies, the lack of a clear mechanism of induction, and the lack of any evidence that inhaled FA even reaches sites distant to the portal of entry.^{1-3,6}

To date, a number of rodent and primate studies have investigated potential modes of action of FA.¹ Its aldehyde moiety readily reacts with nucleophilic sites in proteins and DNA, resulting in DNA-protein and DNA-DNA crosslinks as well as a number of DNA and protein adducts including N^2 -hydroxymethyl-dG (N^2 -HOME-dG) adducts in DNA^{3,6} and FA-induced Schiff bases on lysine residues of proteins.⁷ Our recent studies revealed N^6 -formyllysine (FLys) as an abundant and stable protein adduct caused by FA exposure.^{8,9} FLys formation on cellular proteins, including histones with important epigenetic regulatory roles,^{8,10} and its established association with oxidative and nitrosative stresses of inflammation,^{11,12} suggest that this adduct may play a role in many pathophysiological processes in humans.

With FA as the major source of FLys,^{8,9} we sought to define the contribution of exogenous *versus* endogenous sources of this chemical to the total burden of FLys in cells, using [$^{13}\text{C}^2\text{H}_2$]-FA to distinguish exogenous from endogenous contributions.⁹ An initial *in vivo* study showed a clear concentration-dependent formation of exogenous adducts in nasal

epithelium of rats exposed by inhalation to 0.7–9.1 ppm [$^{13}\text{C}^2\text{H}_2$]-FA for 6 h, with endogenous adducts dominating even at the highest exposure of 9.1 ppm.⁹ However, these studies did not address longer-term exposures of at lower concentrations more relevant to humans. Here, we have investigated the effects of long-term, low-dose inhalation exposure to [$^{13}\text{C}^2\text{H}_2$]-FA on the formation, accumulation, and clearance of FLys adducts in different tissues in rats.

Materials and Methods

Materials

Lysine internal standard, 4,4,5,5,-[^2H]-Lysine, was purchased from Cambridge Isotope Laboratories (Andover, MA) and N^6 -Formyllysine internal standard, 4,4,5,5,-[^2H]- N^6 -formyllysine, was synthesized from 4,4,5,5,-[^2H]-lysine according to Jiang et al.¹² *Streptomyces griseus* protease was obtained from Sigma-Aldrich (St. Louis, MO). Subcellular Protein Fractionation Kit was purchased from Thermo Scientific (Waltham, MA).

Formaldehyde exposure

Fischer 344 rats (6-week old, male) were exposed to [$^{13}\text{C}^2\text{H}_2$]-formaldehyde vapor by single-exposure, nose-only inhalation with the final target exposure concentration of 2 ppm for 7, 14, 21, and 28 d (6h/d). The exposure was done at the Lovelace Respiratory Research Institute (Albuquerque, NM) according to the appropriate and approved protocols for the use of vertebrate animals in experiments. For post-exposure studies, the loss of FLys adducts after 28 d of exposure was investigated with analyses at 6, 24, 72, and 168 h following cessation of FA inhalation. Rats were euthanized using an intraperitoneal barbiturate injection at pre-determined time points. Nasal turbinates were collected by splitting the skull with a slight bias to one side to preserve septal mucosa. Tissue samples were harvested, wrapped in aluminum foil, and immediately snap frozen in liquid nitrogen. They were stored at $-80\text{ }^\circ\text{C}$ pending analyses.

Protein extraction from tissue

Proteins were extracted from tissues as previously described.⁸ Briefly, ~5–10 mg of nasal epithelium, trachea, lung, or bone marrow was cut into small pieces and washed with PBS. Tissues were then homogenized (in cytoplasmic extraction buffer of Subcellular Protein Fractionation Kit) using a Kontes all-glass Dounce homogenizer with 5 strokes of a type A pestle followed by 10 strokes of a type B pestle. Proteins from WBC were also extracted in a similar fashion. For total protein extraction, cell lysate was centrifuged at $14000 \times g$ for 15 min at $4\text{ }^\circ\text{C}$ and supernatant collected. To further examine exogenous and endogenous adduct distribution in different cellular compartments, proteins extracted from nasal epithelium were further separated into cytoplasmic, membrane, soluble nuclear, and chromatin bound fractions using Subcellular Protein Fractionation Kit. Proteins in each fraction were precipitated by adding 20% v/v trichloroacetic acid followed by overnight incubation at $4\text{ }^\circ\text{C}$. Samples were centrifuged at $14000 \times g$ for 10 min at $4\text{ }^\circ\text{C}$, washed with ice-cold acetone (once with acetic acetone containing 0.1% HCl and once more with

acetone), air-dried and kept at -20°C pending analyses. N^6 -Formyllysine was found to be stable under these conditions.

Enzymatic hydrolysis of proteins

Proteins were hydrolyzed into single amino acids as described previously.⁸ Briefly, extracted proteins were dissolved in 50 μL of 100 mM ammonium bicarbonate buffer (pH 8.5). Enzymatic digestion was done by adding 2 μL of freshly prepared *Streptomyces griseus* protease solution (1 $\mu\text{g}/\mu\text{L}$) and incubating at 37°C for 16 h. 2 nmol 4,4,5,5- ^{2}H -lysine and 1 pmol 4,4,5,5- ^{2}H - N^6 -formyllysine internal standards were added prior to enzymatic digestion. Using our methods, samples generally yielded 20–100 μg of protein for which we had confirmed complete proteolysis using 2 μg of *Streptomyces griseus* (based on control studies of theoretical versus measured lysine levels of commercial histone proteins). Regardless, the difference in protein quantities among samples was corrected for by normalizing the quantity of FLys to the quantity of lysine in each analysis. Samples were vacuum dried and resuspended in 50 μL of water prior to mass spectrometry analysis. Typically 1 μL of prepared protein hydrolysates was injected per analysis.

N^6 -Formyllysine quantification

Using sensitive and specific liquid chromatography-coupled mass spectrometry (LC-MS/MS) methods, N^6 -formyllysine was quantified as a percentage of lysine as described previously.⁸ Analytes in each sample were first HPLC separated (Agilent 1100 series system) using an aqueous normal-phase Cogent diamond hydride column (2.1 \times 150 mm, 4 μm particle size) from MicroSolv Technology Corporation (Eatontown, NJ). The solvent systems were 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B), with a flow rate of 400 $\mu\text{L}/\text{min}$ and a temperature of 20°C . With the elution starting at 100% B, the gradient linearly decreased to 75% B over 75 min, reaching 25% B by 78 min, and 15% B by 83 min before re-equilibration with 100% B for 7 min. Mass spectrometry detection and quantification of FLys and lysine were done using Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operated in positive ion mode. ESI capillary voltage was set to 4000 V, gas temperature to 350°C , nebulizer pressure to 30 psi, and drying gas flow to 12 L/min. Selected reaction monitoring (SRM) transitions were m/z 175 \rightarrow 112 for endogenous FLys (neutral loss of H_2O , CO, and NH_3 with an intramolecular rearrangement), m/z 177 \rightarrow 114 for exogenous FLys, and m/z 179 \rightarrow 116 for FLys internal standard (Figure 1). As shown in Figure 1, there is another peak with similar transition as exogenous FLys (m/z 177 \rightarrow 114) that elutes \sim 57.5 min. It is identified as the [M + 1] ion of citrulline and has no association with exogenous formaldehyde.⁸ Lysine and lysine internal standard were monitored with transitions m/z 147 \rightarrow 130 (neutral loss of NH_3), and m/z 151 \rightarrow 134, respectively. The fragmentor voltage and collision energies were 105 V and 10 V for FLys and 100 V and 8 V for lysine, respectively. The limits of detection for lysine and FLys were 10 and 1 fmol, respectively.

Results

Endogenous FLys was detectable in all tissues analyzed, with levels ranging from 1 to 9 adducts per 10^4 lysines depending on the tissue type (Table 1). The endogenous levels in

each tissue were similar across all time points of FA exposure (Table 1), indicating that exogenous FA did not alter the endogenous adduct levels. This is consistent with previous studies at higher FA doses.⁹ There were significant differences in FLys levels among the tissues (WBC \ll nasal < bone < lung < trachea; Table 1), possibly due to different levels of endogenous FA production. To further examine FLys adduct distribution in different cellular compartments, proteins extracted from nasal epithelium were further resolved into cytoplasmic, membrane, and nuclear proteins. Data showed that the endogenous FLys levels were similar among the various cellular compartments (Tables S1 and S2, Supporting Information), which suggests that endogenously-generated FA is freely accessible to all cellular compartments. This differs for exposure to exogenous FA, as discussed next.

Unlike FLys derived from endogenous FA, the levels of FLys caused by exposure to exogenous FA were only detected in nasal epithelium and to some extent in trachea (Table 2, Table S3, Supporting Information), with endogenous FLys ~3- to 18-fold and ~60- to 130-fold higher than exogenous levels, respectively, in total proteins. Exogenous FLys showed a trend toward accumulation over the 4-week exposure period, increasing ~2-fold from day 7 to day 28 (Figure 2, Table 2). However, this trend was not statistically significant (e.g., $p=0.055$ for total proteins).

In tissues distant from the upper airway (lung, bone marrow, white blood cells), FA exposure did not produce a detectable increase in exogenous FLys beyond the natural isotope abundance level of ~0.7%, corresponding to [M+2] ion of FLys (Table 2, Table S3, Supporting Information). This suggests that inhaled FA is mostly absorbed in the nasal passages before reaching distant sites. Intracellular compartments accessibility trend, with exogenous FLys levels generally 2- to 3-fold lower in chromatin (with histones as the major proteins) than other compartments (Figure 2, Table S1, Supporting Information). For example, on Day 28 of FA exposure, there were 0.4 exogenous FLys adducts per 10^4 lysines in chromatin compared to 1.1 residues in cytoplasmic or membrane fractions ($p < 0.05$). This could be due to reaction of FA with biomolecules as it enters the cell before it reaches the nucleus. These compartment biases are likely not a result of differential removal or repair mechanisms since endogenous FLys levels are similar in all compartments.

To assess the decay of FLys in FA-exposed tissues, we quantified FLys levels in tissues and subcellular compartments at 6, 24, 72 and 168 h after 28 d of FA exposure (Table 2; Tables S2, S3, Supporting Information). In all cases, there was a bi-exponential decay pattern for FLys levels, with an initial rapid loss over 24 h ($t_{1/2}$ of ~18–34 h) followed by a slower decay ($t_{1/2}$ of ~151–186 h) over the next 6 d (Figure 3; Figure S1, Supporting Information).

Discussion

A parallel investigation of FA-induced N^2 -hydroxymethyl-dG (N^2 -HOMe-dG) and DNA-protein cross-links in the same study group revealed strong correlations between the DNA damage^{13,14} and FLys. Similar to exogenous FLys, exogenous N^2 -HOMe-dG and DNA-protein cross-links showed formation and accumulation in nasal epithelium and not in tissues distant to the site of initial contact.^{13,14} Furthermore, similar to FLys, the exogenous N^2 -HOMe-dG adducts and DNA-protein cross-links occurred at lower levels than

endogenous species (~2- to 7- fold) at all exposure time points. However, while FLys and *N*²-HOMe-dG exhibited a measurable late elimination half-life (151–186 h and 170 h, respectively), the DNA-protein cross-links persisted at relatively high levels beyond 7 d post-exposure.¹⁴

These differences in formation and clearance of FA adducts provide insights into the molecular basis of FA toxicity and carcinogenicity. The consistent observation of FA-induced protein and nucleic acid adducts only in the most immediately exposed tissues, and not at sites distant to the portal of entry (e.g., bone marrow), raises questions about the proposed link between inhaled FA and leukemia.^{1,2,4} At the same time, the diversity of FA-induced molecular damage both complicates and reinforces conclusions about mode of action of FA as a carcinogen. For example, FLys formation at conserved sites of lysine acetylation and methylation in histone proteins,¹⁰ the chemical similarity of FLys and lysine *N*⁶-acetylation,⁸ and the resistance of FLys adducts to removal by histone deacetylases⁸ all suggest that FLys could interfere with histone-based epigenetics¹⁵ as one potential mechanism of toxicity and carcinogenesis. Such epigenetic effects could combine with the direct genotoxicity of FA-induced DNA adducts and DNA-protein cross-links as well as indirect genotoxicity of mutagenesis associated with FA-induced cell death, to cause mutations and cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

This project was funded by the MIT David H. Koch Cancer Research Fund and the NIH/NIEHS (grants ES016450, ES005948, ES010126, ES002109), NIH/NCI (grants CA026731, CA103146), and the Texas Commission for Environmental Quality. LC-MS studies were performed in the NIEHS-supported MIT Center for Environmental Health Sciences. Formaldehyde exposures were supported by the Research Foundation for Health and Environmental Effects, a 501 (c)(3) organization.

Abbreviations List

FA	formaldehyde
FLys	<i>N</i> ⁶ -formyllysine
LC-MS/MS	liquid chromatography-coupled tandem quadrupole mass spectrometry
WBC	white blood cells
<i>N</i>²-HOMe-dG	<i>N</i> ² -hydroxymethyl-dG

References

1. Swenberg JA, Moeller BC, Lu K, Rager JE, Fry RC, Starr TB. Formaldehyde carcinogenicity research: 30 years and counting for mode of action, epidemiology, and cancer risk assessment. *Toxicologic pathology*. 2013; 41:181–189. [PubMed: 23160431]
2. Humans IWGotEoCRt. Chemical agents and related occupations. IARC monographs on the evaluation of carcinogenic risks to humans/World Health Organization, International Agency for Research on Cancer. 2012; 100:9–562.
3. Moeller BC, Lu K, Doyle-Eisele M, McDonald J, Gigliotti A, Swenberg JA. Determination of N2-hydroxymethyl-dG adducts in the nasal epithelium and bone marrow of nonhuman primates following 13CD2-formaldehyde inhalation exposure. *Chemical research in toxicology*. 2011; 24:162–164. [PubMed: 21222454]
4. Zhang L, Freeman LE, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR. Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. *Environ Mol Mutagen*. 2010; 51:181–191. [PubMed: 19790261]
5. Pontel LB, Rosado IV, Burgos-Barragan G, Garaycochea JI, Yu R, Arends MJ, Chandrasekaran G, Broecker V, Wei W, Liu L, Swenberg JA, Crossan GP, Patel KJ. Endogenous Formaldehyde Is a Hematopoietic Stem Cell Genotoxin and Metabolic Carcinogen. *Molecular cell*. 2015; 60:177–188. [PubMed: 26412304]
6. Lu K, Moeller B, Doyle-Eisele M, McDonald J, Swenberg JA. Molecular dosimetry of N2-hydroxymethyl-dG DNA adducts in rats exposed to formaldehyde. *Chemical research in toxicology*. 2011; 24:159–161. [PubMed: 21155545]
7. Lu K, Boysen G, Gao L, Collins LB, Swenberg JA. Formaldehyde-induced histone modifications in vitro. *Chemical research in toxicology*. 2008; 21:1586–1593. [PubMed: 18656964]
8. Edrissi B, Taghizadeh K, Dedon PC. Quantitative analysis of histone modifications: formaldehyde is a source of pathological n(6)-formyllysine that is refractory to histone deacetylases. *PLoS genetics*. 2013; 9:e1003328. [PubMed: 23468656]
9. Edrissi B, Taghizadeh K, Moeller BC, Kracko D, Doyle-Eisele M, Swenberg JA, Dedon PC. Dosimetry of N(6)-formyllysine adducts following [(1)(3)C(2)H(2)]-formaldehyde exposures in rats. *Chemical research in toxicology*. 2013; 26:1421–1423. [PubMed: 24087891]
10. Wisniewski JR, Zougman A, Mann M. Nepsilon-formylation of lysine is a widespread post-translational modification of nuclear proteins occurring at residues involved in regulation of chromatin function. *Nucleic Acids Res*. 2008; 36:570–577. [PubMed: 18056081]
11. Vana L, Kanaan NM, Hakala K, Weintraub ST, Binder LI. Peroxynitrite-induced nitrative and oxidative modifications alter tau filament formation. *Biochemistry*. 2011; 50:1203–1212. [PubMed: 21210655]
12. Jiang T, Zhou X, Taghizadeh K, Dong M, Dedon PC. N-formylation of lysine in histone proteins as a secondary modification arising from oxidative DNA damage. *Proc Natl Acad Sci U S A*. 2007; 104:60–65. [PubMed: 17190813]
13. Yu R, Lai Y, Hartwell HJ, Moeller BC, Doyle-Eisele M, Kracko D, Bodnar WM, Starr TB, Swenberg JA. Formation, Accumulation, and Hydrolysis of Endogenous and Exogenous Formaldehyde-Induced DNA Damage. *Toxicological sciences: an official journal of the Society of Toxicology*. 2015; 146:170–182. [PubMed: 25904104]
14. Lai Y, Yu R, Hartwell HJ, Moeller BC, Bodnar WM, Swenberg JA. Measurement of Endogenous versus Exogenous Formaldehyde-Induced DNA-Protein Crosslinks in Animal Tissues by Stable Isotope Labeling and Ultrasensitive Mass Spectrometry. *Cancer research*. 2016; 76:2652–2661. [PubMed: 26984759]
15. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 2000; 403:41–45. [PubMed: 10638745]

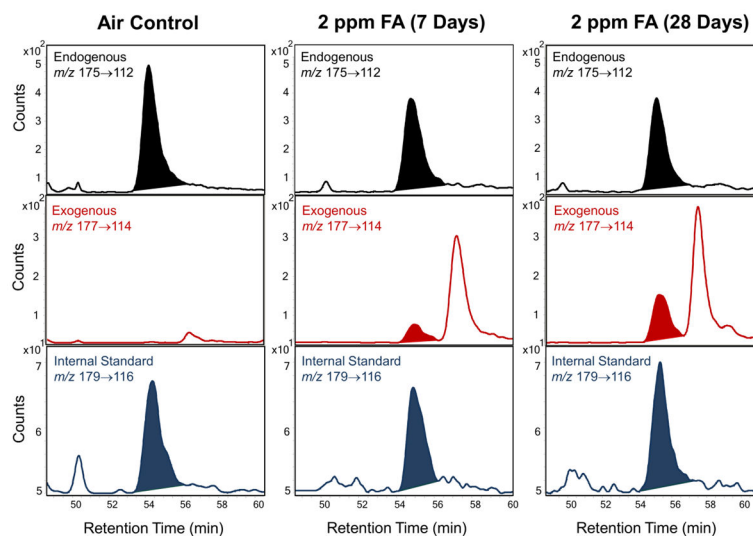


Figure 1.

Accumulation of exogenous N^6 -formyllysine adducts in total protein extracted from nasal epithelium of rats exposed to $[^{13}\text{C}^2\text{H}_2]$ -formaldehyde by inhalation. The use of $[^{13}\text{C}^2\text{H}_2]$ -formaldehyde allows for differentiation of exogenous from endogenous adducts. The three isotopomeric species monitored by LC-MS/MS. Endogenous, exogenous, and internal standard 4,4,5,5- $[^2\text{H}]$ -FLys were monitored by LC-MS/MS using transitions of m/z 175 \rightarrow 112, m/z 177 \rightarrow 114, and m/z 179 \rightarrow 116, respectively.

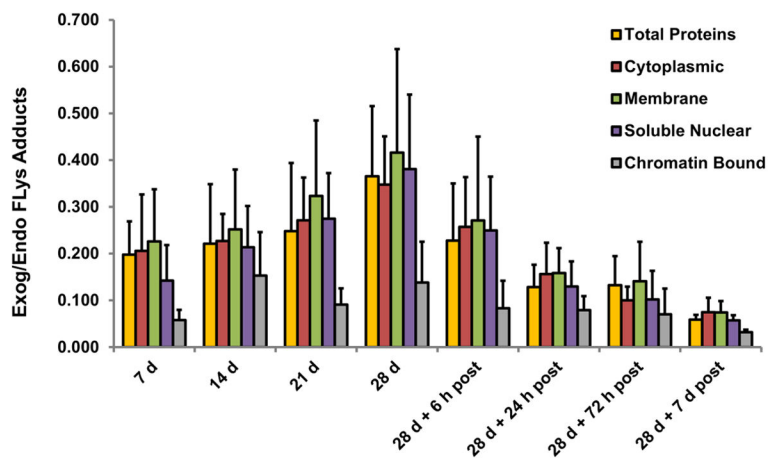


Figure 2. Accumulation and loss of exogenous FLYs adducts. Ratios of exogenous vs. endogenous FLYs in the nasal epithelium of rats exposed by inhalation to 2 ppm [$^{13}\text{C}^2\text{H}_2$]-FA for 7, 14, 21, and 28 days (6h/day). Data represent mean \pm SD for n=4.

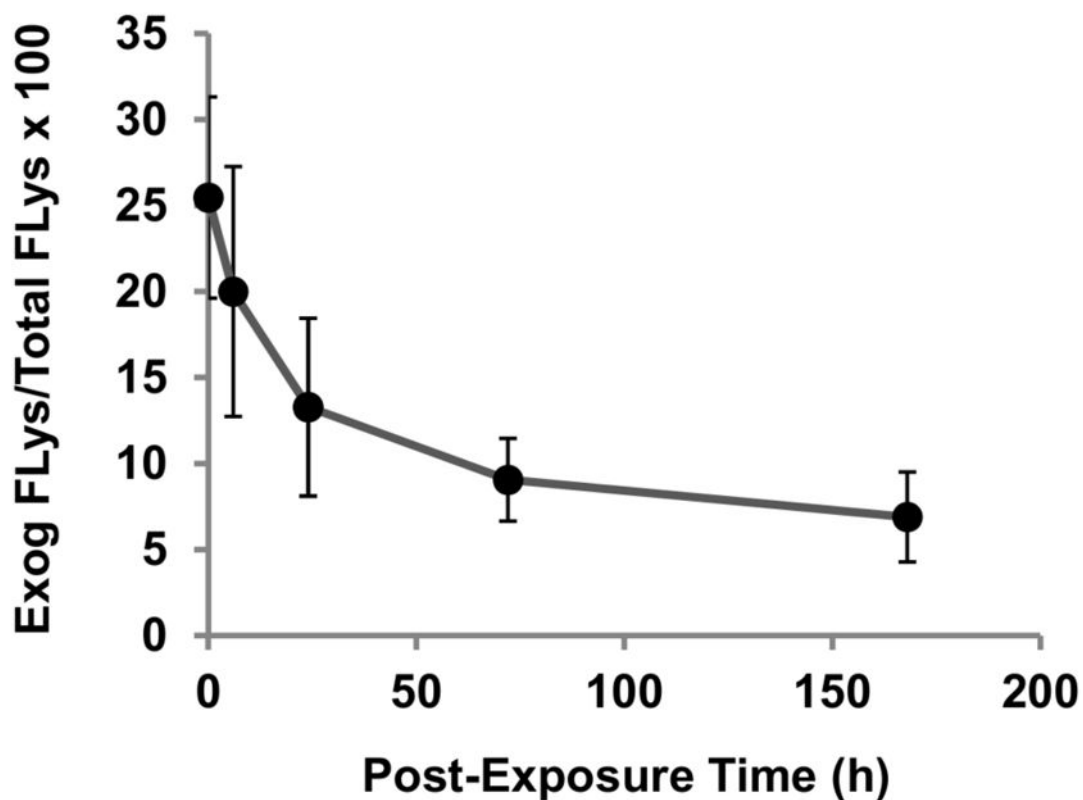


Figure 3. Example of the bi-exponential decay of exogenous FLYs adducts. Exogenous FLYs were quantified in cytoplasmic proteins extracted from nasal epithelium of rats at various times following a 28-day inhalation exposure to 2 ppm [$^{13}\text{C}^2\text{H}_2$]-FA (6h/d). Data show an initial rapid loss over 24 h ($t_{1/2}$ of ~25 h) followed by a slower decay ($t_{1/2}$ of ~182 h) over the next 6 d. Data represent mean \pm SD for n=4.

Table 1

Quantification of endogenous N^6 -formyllysine in total proteins extracted from different tissues of rats exposed by inhalation to 2 ppm [$^{13}C^2H_2$]-formaldehyde (6 h/day).¹

Exposure	Air Control	7 d	14 d	21 d	28 d	28 d + 6 h post	28 d + 24 h post	28 d + 72 h post	28 d + 7 d Post	Mean \pm SD ³
Nasal Epithelium	6.1 \pm 1.0 ²	3.3 \pm 0.4	3.7 \pm 1.1	3.9 \pm 0.4	3.0 \pm 1.3	4.7 \pm 2.1	3.5 \pm 0.9	3.9 \pm 1.0	4.1 \pm 1.2	4.0 \pm 0.9
Trachea	4.0 \pm 0.3	4.9 \pm 1.7	6.9 \pm 3.2	6.0 \pm 2.3	5.7 \pm 1.3	8.1 \pm 4.2	8.9 \pm 2.5	7.1 \pm 4.6	6.7 \pm 1.0	6.5 \pm 1.5*
Lung	3.4 \pm 0.2	6.4 \pm 0.6	5.1 \pm 0.9	5.4 \pm 1.7	3.2 \pm 0.2	7.7 \pm 0.8	8.3 \pm 2.8	6.9 \pm 1.9	8.3 \pm 1.3	6.1 \pm 1.9**
Bone Marrow	4.4 \pm 2.8	3.9 \pm 0.5	4.0 \pm 2.3	4.7 \pm 2.1	5.9 \pm 0.2	4.3 \pm 1.9	4.7 \pm 2.6	4.3 \pm 1.6	3.8 \pm 1.4	4.4 \pm 0.6
WBC	1.1 \pm 0.3	2.3 \pm 2.3	2.4 \pm 1.6	1.3 \pm 1.2	1.6 \pm 0.9	1.3 \pm 0.7	1.6 \pm 1.1	1.3 \pm 0.7	0.8 \pm 0.1	1.5 \pm 0.5***

¹ Endogenous ($^{12}C^1H_2$) N^6 -formyllysine values are expressed as adducts per 10^4 lysines.

² Data represent mean \pm SD for 3 rats (n=4 for nasal epithelium).

³ Data represent the average of all time points \pm SD; *, **, ***, **** significantly different from nasal epithelium by Student's t-test; *, p<0.002; **, p<0.002; ***, p<0.00001.

Relative quantities of exogenous vs. endogenous FLys adducts in total proteins extracted from tissues of rats exposed by inhalation to 2 ppm [$^{13}\text{C}_2\text{H}_2$]-formaldehyde (6 h/d).¹

Table 2

Exposure	7 d	14 d	21 d	28 d	28 d + 6 h post	28 d + 24 h post	28 d + 72 h post	28 d + 7 d post
Nasal Epithelium	20 ± 7 ²	22 ± 13	25 ± 15	37 ± 15	23 ± 12	13 ± 5	13 ± 6	6 ± 1
Trachea	1.5 ± 0.6	1.2 ± 0.1	1.6 ± 0.8	1.4 ± 0.2	1.0 ± 0.1	1.2 ± 0.3	1.1 ± 0.3	0.8 ± 0.3
Lung	< 0.7 ³	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7
Bone Marrow	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7
WBC	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7	< 0.7

¹Data are expressed as the ratio exogenous ($^{13}\text{C}_2\text{H}_2$)/endogenous ($^{12}\text{C}_1\text{H}_2$) x 100 for each rat.

²Data represent mean ± SD for 3 rats (n=4 for nasal epithelium).

³Not detected beyond the natural isotope abundance level of ~0.7% for [M+2] ion of FLys (limit of detection of 1 fmol).