**Supplementary METHODS**

**Synthesis of dG-Me-Cys standard.**

A synthetic standard, dG-Me-Cys, was synthesized by digestion of dG-Me-GSH according to a previous study (1). First, dG-Me-GSH was prepared using formaldehyde to crosslink glutathione and dG. Briefly, glutathione (85 mM) was incubated with formaldehyde (100 mM) in 1.75 ml sodium phosphate buffer (100 mM, pH 7.2) at 37 °C for 4 h before adding a final concentration of 16 mM dG. The crosslinking reaction was performed at 37°C for 6 h. dG-Me-GSH was purified from the reaction mixture by using a C18 reverse phase column (Waters Atlantis T3, 3 µm, 150 mm × 4.6 mm, Milford, MA) equipped on an Agilent 1200 series UV HPLC system. The mobile phases consisted of 0.05% acetic acid in water (A) and pure acetonitrile (B). The column temperature was set at 15°C. The flow rate was 0.45 ml/min and elution gradient conditions were set as follows: 0 min, 2% B; 3 min, 2% B; 20 min, 15% B; 28 min, 15% B; 35 min, 80% B; 35.5 min, 2% B; 45 min, 2% B. dG-Me-GSH was monitored using a wavelength at 254 nm and eluted at the retention time of 24.5 min. The fractions containing dG-Me-GSH were combined and dried in a vacuum concentrator. Subsequently, dG-Me-Cys was synthesized by digestion of dG-Me-GSH using leucine aminopeptidase M and carboxypeptidase Y. Briefly, the isolated dG-Me-GSH was digested in 1.2 ml sodium phosphate buffer (40 mM, pH 6.0) containing 33 µg/ml carboxypeptidase Y, 133 µg/ml leucine aminopeptidase M, 10 mM MgCl2, and 10 mM CaCl2. The digestion reaction was carried out at room temperature for 16 h. Enzymes were removed by a Nanosep Centrifugal Device (MWCO 3K) with spin speed at 11,000 × g for 30 min at 4°C. Separation of dG-Me-Cys was performed using the same HPLC method as described above with a different elution gradient. The elution gradient conditions were set as follows: 0 min, 2% B; 3 min, 2% B; 42 min, 4.1% B; 43 min, 90% B; 55 min, 90% B; 55.5 min, 2% B; 65 min, 2% B. dG-Me-Cys eluted at the retention time of 38.5 min. The fractions containing dG-Me-Cys were collected and concentrated in a vacuum concentrator.

**Enzyme Wash.**

In order to eliminate matrix interferences caused by self-digestion, pronase (20 mg/ml) was incubated at 55 °C for 10 min, followed by incubation at 37 °C for 1 hour. The treated pronase was washed using an Amicon Ultra Centrifugal Filter (MWCO, 3K). Specifically, 150 µl of treated pronase was mixed with 350 µl of wash solution (5 mM MgCl2, 5 mM CaCl2) in a centrifugal filter, followed by centrifugation at 11,000 × g and 4 °C for 13 min. This wash step was repeated two more times. Additionally, 150 µl of the enzyme mixture consisting of carboxypeptidase Y (0.3 mg/ml), aminopeptidase M (0.6 mg/ml), and prolidase (0.3 mg/ml) was washed using this same technique prior to use.

**Animal exposures.**

Animal use in this study was approved by the Institutional Animal Use and Care Committee of The Lovelace Respiratory Research Institute (LRRI) and was conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. Animals were housed in fully accredited American Association for Accreditation of Laboratory Animal Care facilities. All animal exposures were conducted at LRRI in Albuquerque, NM. Animals were observed twice daily from arrival at the facility through necropsy. Animals were weighed prior to the start of the study for randomization. Temperature (18-26 °C) and relative humidity (30-70%) in animal housing areas were within the target range specified in the protocol throughout the quarantine and dosing periods. The F344 male rats were obtained from Charles River Laboratories and were between 8-12 weeks of age at the initiation of exposures. The male cynomolgus macaques (*Macaca fasicularis*) were obtained from the LRRI colony and were between 2.7 – 4.7 years of age with weights ranging from 2.4 to 5.8 kg. All animals were conditioned to the exposure chambers using house air before the initiation of the experiment.

[13CD2]-Formaldehyde was generated by the thermal depolymerization of [13CD2]-paraformaldehyde (CAS No. 30525-89-4) obtained from Cambridge Isotopes Laboratories, Inc (Anover, MA). The inhalation exposure system consisted of a vapor generated source and an exposure plenum (chamber). The test atmospheres of [13CD2]-formaldehyde were generated from an 100 liter Tedlar bag using a peristaltic pump to deliver vaporized [13CD2]-formaldehyde into the chamber supply airflow using a stainless steel vapor delivery line diluted with filtered house air. Oxygen levels (%) were monitored throughout the exposure. During each of the exposures, formaldehyde inside of the Tedlar® bag was diluted with pre-filtered air and delivered into the exposure plenum to achieve the target formaldehyde concentration of either 2.0 or 15.0 ppm. The time to 90% vapor concentration for the whole body exposures was <10 minutes and chamber homogeneity was <5% relative standard deviation at the sampling ports. Chambers were maintained at a slightly negative pressure relative to the exposure room. Control animals were exposed to filtered air using nose-only exposure chambers.

The concentration of the exposure chamber was monitored by collection of Waters XpoSure Aldehyde Sampler cartridges approximately every hour for rats and every 30 minutes for nonhuman primates (NHPs) throughout the exposure. Cartridges were extracted with acetonitrile and extracts were analyzed by HPLC-UV-MS monitoring the 2,4-Dinitrophenylhydrazine (DNPH) derivative, DNPH-formaldehyde, using an Agilent 1100 HPLC coupled to a PE Sciex API 365 (EP 10 upgrade) triple quadrupole mass spectrometer utilizing a 50:50 isocratic flow of 0.05M acetic acid in (A) purified water and (B) acetonitrile. UV wavelength detection was set at 360 nm. A Zorbax XDB-C18, 5 µ, 2.1 x 150 mm column was used and retention time for DNPH derivatized formaldehyde was 5.5 minutes. Standards were prepared from formaldehyde 2,4-dinitrophenylhydrazone purchased from Sigma-Aldrich. Linear range used was from 1.5 to 100 µg/ml. The stable isotope labeled [13CD2]-DNPH-formaldehyde (214 *m/z*) was confirmed by full scan mass spectrometry collected from 100 – 400 *m/z*. Unlabeled formaldehyde-DNPH derivatives had a mass of 211 *m/z.*

For the 2 ppm rat study, male F344 rats were exposed to 2 ppm [13CD2]-formaldehyde atmospheres for either 7 or 28 consecutive days (6 h/day) with post exposure at 24 and 168 hours using a nose-only exposure system (Supplementary Fig. S2). For the 15 ppm rat study, male F344 rats were exposed to 15 ppm [13CD2]-formaldehyde atmospheres for either 1, 2, or 4 consecutive days (6 h/day) using a nose-only exposure system. The actual chamber concentrations for both groups are shown in the supporting information (Supplementary Table S2 and S3). Following exposures, at predetermined time points, rats were euthanized following the administration of a pentobarbital-based euthanasia solution (IP) to induce surgical-level anesthesia and pneumothorax was introduced. Heparinized blood was drawn prior to necropsy. The blood was processed using CPT tubes to selectively collect PBMC from blood. Tissues and PBMC were snap frozen in liquid nitrogen or dry ice/ethanol slurry prior to storage at -80 oC.

For the 6 ppm NHP study, cynomolgus macaques were whole body exposed to 6 ppm [13CD2]-formaldehyde for 2 consecutive days (6 h/day) using a whole body exposure system (Supplemental Fig. S3). During the 6 hour exposures, Tedlar bags (112-1198 ppm) were directed into the whole body chamber through a supply pump running a total flow of ~ 1.5 L/min with chamber exhaust flows ranging from 244 to 265 l/min. The exposures were staggered over a 6-day period with an average aerosol concentration of 6.2 ppm with a standard deviation of 1.9 (Supplementary Table S4). The first two days of exposure had much larger standard deviation as compared to the last 4 days.

NHPs were sedated with Ketamine (10 mg/kg, IM) and administered Euthasol within 2 h following the cessation of exposure. Immediately after the animal was anesthetized, 3-5 ml of blood was collected for PBMC isolation as described for rats. Nasal respiratory epithelium from the right and left sides of the nose and from the septum were collected, as was the liver. Bone marrow was collected from both femurs by saline extrusion with a large bore needle. Tissue samples, including bone marrow and PBMC were collected and immediately frozen with liquid nitrogen followed by storage at -80 °C.

**Reference**

(1) Yu R, Lai Y, Hartwell HJ, Moeller BC, Doyle-Eisele M, Kracko D, et al. Formation, accumulation and hydrolysis of endogenous and exogenous formaldehyde induced DNA damage. Toxicol Sci 2015;146(1):170-82.