Endogenous Chemical Risk Assessment: Formaldehyde as a Case Example

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Formaldehyde is One of the Oldest Chemicals in the World

Formaldehyde was Part of the Origin of Life

Sources of Endogenous Formaldehyde

- One-carbon pool
- Methanol metabolism
- Amino Acid metabolism
- Lipid Peroxidation
- P450 dependent demethylation (O-, N-, S-methyl)
Ubiquitous Environmental Chemical

- Global production is >20 million tons/yr
- Wide use in industrial and consumer products
- Carcinogenic in rodent bioassays
- Listed as a human carcinogen
  - NTP 2011, IARC 2006
- Mode of Action is complex
  - Cytotoxic/cell proliferation
  - Mutagenic
  - Site of contact vs distant sites
  - Endogenously formed in all cells
Epidemiology of Formaldehyde and Cancer

• Nasopharyngeal Cancer
  – The NCI cohort found an increase in NPC, while other studies have been negative.
    • Only 1 plant out of 10 had an increased incidence of NPC
    • The same plant was in a region known for silversmithing and metal working, two known causes of NPC.
    • The extent of formaldehyde exposure was not associated with the increase in NPC.

• While biologic plausibility is clearly present, the lack of consistency between studies and the lack of an exposure relationship in positive studies weakens the conclusion.

• Confounding cannot be eliminated.
Epidemiology of Formaldehyde and Cancer (Cont.)

• Myeloid Leukemia
  – No evidence has been provided that demonstrates that formaldehyde gets to sites distant to the portal of entry.
  – While several studies have shown associations, equal numbers of studies have not.
  – No mechanisms have been identified for the induction of leukemia by formaldehyde.
  – Thus, the biologic plausibility of inhaled formaldehyde causing leukemia is weak.
Carcinogenesis Bioassays

• CIIT/Battelle studies in rats and mice
  – 12 month sacrifice/interim report

• CIIT expanded the exposure range and mechanistic designs in a second bioassay published in Cancer Research (Monticello, et al, 1996)

• Subsequent cancer bioassays
  – Inhalation studies
  – Oral studies
Tumor Incidence and Cell Proliferation in Rats Exposed to Formaldehyde

HCHO Concentration (ppm)

Tumor Incidence (%)

Cell Proliferation (mean unit length labeling index) at Nasal Level II (fold increase over control)

- Tumor Incidence 24-month Study (Kerns, 1983)
- Tumor Incidence 24-month Study (Monticello, 1996)
- Cell Proliferation Study 6-month (Monticello, 1990)
- Cell Proliferation Study 12-month (Monticello, 1990)
- Cell Proliferation Study 18-month (Monticello, 1990)
Early Mode of Action Studies

• Cytotoxicity and cell proliferation studies
  – Focused on short term exposures and CxT
  – Culminated with the Monticello study with 6, 12 and 18 month exposures for cell proliferation

• Minute volume studies comparing rats and mice
  – Mice reduce respiratory minute volume so a 15 ppm exposure is similar to a 6 ppm exposure in rats

• Mucocilliary clearance

• Airflow modeling in rats, primates and humans
Early Mode of Action Studies

• DNA-protein cross-link quantitation
  – Careful assays based on physical chemistry were conducted in rats and primates
  – Demonstrated nonlinear exposure relationships
  – Did not find any accumulation in multiple day exposures
    • Methods could not distinguish between loss, repair and protease degradation down to peptides
    • Methods could not distinguish between exogenous and endogenous formaldehyde cross-links
DNA-Protein Cross-links versus FA Exposure

# Breathing Patterns

Concentrations of DNA–protein cross-links in the respiratory tract, sinuses, and bone marrow (Femur) of Rhesus monkeys exposed to $[^{14}C]$formaldehyde$^d$

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of DNA–protein cross-links (pmol/mg DNA)$^b,c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.7 ppm</td>
</tr>
<tr>
<td>Middle turbinates + lateral wall/septum$^d$</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>0.09 ± 0.09</td>
</tr>
<tr>
<td>Larynx/trachea/carina</td>
<td>ND$^e$</td>
</tr>
<tr>
<td>Airways$^f$</td>
<td>ND$^e$</td>
</tr>
<tr>
<td>Sinuses</td>
<td>ND$^e$</td>
</tr>
<tr>
<td>Proximal lung</td>
<td>ND$^e$</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>ND$^e$</td>
</tr>
</tbody>
</table>


![Lesion Distribution](image)

Kimbell et al. Toxicol. Sci. 64, 100-110 (2001) Figure 5.
Recent Molecular Mode of Action Studies

Formaldehyde is very reactive with proteins and DNA, leading to diverse protein adducts and DNA damage.
Formaldehyde Specific DNA Adducts

13CD2O Exposure → Tissue Collection → DNA Isolation → Reduction with NaCNBH3 → Digestion and HPLC Fractionation → Nano-LC-MS/MS
LC-ESI-MS/MS SRM chromatograms of N2-Me-dG in typical tissues: 1 day-exposed nasal epithelium (A), 5 day-exposed nasal epithelium (B), bone marrow (C) and spleen (D).
Formaldehyde-induced $N^2$-hydroxymethyl-dG adducts in rats exposed to 10 ppm Formaldehyde for 1 or 5 days

<table>
<thead>
<tr>
<th>Exposure Period</th>
<th>Tissues</th>
<th>Exogenous adducts/10$^7$ dG</th>
<th>Endogenous adducts/10$^7$ dG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>Nose</td>
<td>$1.28 \pm 0.49$</td>
<td>$2.63 \pm 0.73$</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>nd</td>
<td>$2.39 \pm 0.16$</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>nd</td>
<td>$2.66 \pm 0.53$</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>nd</td>
<td>$2.35 \pm 0.31$</td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>nd</td>
<td>$1.05 \pm 0.14$</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>nd</td>
<td>$2.19 \pm 0.36$</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>nd</td>
<td>$1.28 \pm 0.38$</td>
</tr>
<tr>
<td>5 day</td>
<td>Nose</td>
<td>$2.43 \pm 0.78$</td>
<td>$2.84 \pm 1.13$</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>nd</td>
<td>$2.61 \pm 0.35$</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>nd</td>
<td>$3.24 \pm 0.42$</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>nd</td>
<td>$2.35 \pm 0.59$</td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>nd</td>
<td>$1.17 \pm 0.35$</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>nd</td>
<td>$1.99 \pm 0.30$</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>nd</td>
<td>$1.10 \pm 0.28$</td>
</tr>
</tbody>
</table>
Improved Methodology

• LOD: 20 attomoles
• LOQ: 40 attomoles

Instrumentation

– Waters NanoAcquity UPLC
  • Waters C18 T3 Nano
  • Flow Rate: 0.6 µL/min
  • 24 minute reverse phase gradient
  • Mobile Phases:
    – A) Water with 0.1% Acetic Acid
    – B) ACN with 0.1 % Acetic Acid
  
– Thermo Quantum Ultra Triple Quadrupole MS
  • Scan Speed: 0.1 seconds per transition
  • Collision Energy: 17 eV
  • Peak Width
    – Q1: 0.3 dalton
    – Q3: 0.5 dalton
  • Scan Width: 1 dalton
  • ESI nano source – positive mode

20 amol on column
LOD is about 10 amol
# Dosimetry of N²-hydroxymethyl-dG Adducts in Nasal Epithelium of Rats

<table>
<thead>
<tr>
<th>Exposure (ppm)</th>
<th>Exogenous adducts/10⁷ dG</th>
<th>Endogenous adducts/10⁷ dG</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7±0.2</td>
<td>0.039±0.019</td>
<td>3.62±1.33</td>
<td>3*</td>
</tr>
<tr>
<td>2.0±0.1</td>
<td>0.19±0.08</td>
<td>6.09±3.03</td>
<td>4**</td>
</tr>
<tr>
<td>5.8±0.5</td>
<td>1.04±0.24</td>
<td>5.51±1.06</td>
<td>4</td>
</tr>
<tr>
<td>9.1±2.2</td>
<td>2.03±0.43</td>
<td>3.41±0.46</td>
<td>5</td>
</tr>
<tr>
<td>15.2±2.1</td>
<td>11.15±3.01</td>
<td>4.24±0.92</td>
<td>5</td>
</tr>
</tbody>
</table>

*4-6 rats combined
** 2 rats combined
Ratio of Exogenous to Endogenous Adducts

Exogenous

Endogenous

Formaldehyde Exposure Dose (ppm)

Ratio of Exogenous Versus Endogenous Adducts
Non-Human Primate Study

• $^{13}$CD$_2$O Exposure for 2 days (6 hours/day) at 2 or 6 ppm (n=4)

• Cynomolgus Macaque

• Tissues (to date)
  – Nasal turbinates
  – Femoral Bone Marrow
  – Brain
  – Lung
Adduct Numbers in Primate Nasal
Maxilloturinbates

<table>
<thead>
<tr>
<th>Exposure concentration</th>
<th>Exogenous adducts/$10^7$ dG</th>
<th>Endogenous adducts/$10^7$ dG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 ppm</td>
<td>0.25 ± 0.04</td>
<td>2.49 ± 0.39</td>
</tr>
<tr>
<td>6.1 ppm</td>
<td>0.41 ± 0.05</td>
<td>2.05 ± 0.53</td>
</tr>
</tbody>
</table>

n = 3 or 4
Primate Femoral Bone Marrow Endogenous and Exogenous Adducts

1.9 ppm $^{13}$CD$_2$O  
6.1 ppm $^{13}$CD$_2$O

312 µg DNA

178 µg DNA

No Exogenous Adducts Detected with 5-10 fold >DNA

Note: We used ~20-30 µg for nasal tissue
### Adduct Numbers in Primate Bone Marrow

<table>
<thead>
<tr>
<th>Exposure concentration</th>
<th>Exogenous adducts/10^7 dG</th>
<th>Endogenous adducts/10^7 dG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 ppm</td>
<td>nd</td>
<td>17.48 ± 2.61</td>
</tr>
<tr>
<td>6.1 ppm</td>
<td>nd</td>
<td>12.45 ± 3.63</td>
</tr>
</tbody>
</table>

n = 4
Recent Improvements in Methodology

- **Instrumentation**
  - SCIEX 6500 Triple Quadrupole MS
- **LOD**: 1.5 attomoles
- **LOQ**: 4 attomoles
**N²-Methyl-dG Adducts in Rat Nasal Epithelium Following 2 ppm Exposure for up to 28 days (6 hr/day)**

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Exogenous adducts/10⁷ dG</th>
<th>Endogenous adducts/10⁷ dG</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 day</td>
<td>0.35 ± 0.17</td>
<td>2.51 ± 0.63</td>
<td>5</td>
</tr>
<tr>
<td>14 day</td>
<td>0.84 ± 0.17</td>
<td>3.09 ± 0.98</td>
<td>5</td>
</tr>
<tr>
<td>21 day</td>
<td>0.95 ± 0.11</td>
<td>3.34 ± 1.06</td>
<td>5</td>
</tr>
<tr>
<td>28 day</td>
<td>1.07 ± 0.16</td>
<td>2.82 ± 0.76</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 6 hr</td>
<td>0.85 ± 0.38</td>
<td>2.61 ± 0.55</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 24 hr</td>
<td>0.83 ± 0.61</td>
<td>2.87 ± 0.65</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 72 hr</td>
<td>0.64 ± 0.14</td>
<td>2.95 ± 0.71</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 168 hr</td>
<td>0.76 ± 0.19</td>
<td>2.69 ± 0.45</td>
<td>6</td>
</tr>
</tbody>
</table>
**N²-hydroxymethyl-dG Adduct Half-life Study**

\[ t_{1/2} = 63 \text{ hours} \]

**Graph Details:**
- **Graph Title:** N²-hydroxymethyl-dG Adduct Half-life Study
- **Equation:** \( Y = -0.011x - 0.46 \)
- **R² Value:** 0.771
- **Data Points:** n=5 per time point
- **Mean ± SD:**

**Graph Description:**
- The graph shows the natural logarithm of exogenous adducts per 10⁷ dG against hours.
- The trend line indicates a decreasing number of adducts over time.
- The equation and R² value provide a measure of the goodness of fit for the model.
### $N^2$-Methyl-dG Adduct Numbers in Rat Bone Marrow Following 2 ppm Exposure for up to 28 days (6 hr/day)

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Exogenous adducts/$10^7$ dG</th>
<th>Endogenous adducts/$10^7$ dG</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 day</td>
<td>nd</td>
<td>3.37 ± 1.56</td>
<td>6</td>
</tr>
<tr>
<td>14 day</td>
<td>nd</td>
<td>2.72 ± 1.36</td>
<td>6</td>
</tr>
<tr>
<td>21 day</td>
<td>nd</td>
<td>2.44 ± 0.96</td>
<td>6</td>
</tr>
<tr>
<td>28 day</td>
<td>nd</td>
<td>4.06 ± 3.37</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 6 hr</td>
<td>nd</td>
<td>2.41 ± 1.14</td>
<td>6</td>
</tr>
<tr>
<td>28 day + 24 hr</td>
<td>nd</td>
<td>4.67 ± 1.84</td>
<td>5</td>
</tr>
<tr>
<td>28 day + 72 hr</td>
<td>nd</td>
<td>5.55 ± 0.76</td>
<td>6</td>
</tr>
<tr>
<td>28 day + 168 hr</td>
<td>nd</td>
<td>2.78 ± 1.94</td>
<td>4</td>
</tr>
</tbody>
</table>
**$N_2$-Methyl-dG Adduct Numbers in Rat WBC Following 2 ppm Exposure for up to 28 days (6 hr/day)**

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Exogenous adducts/10^7 dG</th>
<th>Endogenous adducts/10^7 dG</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 day</td>
<td>nd</td>
<td>4.91 ± 3.71</td>
<td>4</td>
</tr>
<tr>
<td>14 day</td>
<td>nd</td>
<td>3.01 ± 0.54</td>
<td>4</td>
</tr>
<tr>
<td>21 day</td>
<td>nd</td>
<td>3.53 ± 0.72</td>
<td>4</td>
</tr>
<tr>
<td>28 day</td>
<td>nd</td>
<td>3.53 ± 0.72</td>
<td>4</td>
</tr>
</tbody>
</table>
New Research Studies

• Epigenetic effects of inhaled formaldehyde.
  – EHP paper for epigenetic studies in monkey maxilloturbinate.
  – 1 and 4 week exposures to 2 ppm formaldehyde and 1 week post exposure show changes in nasal tissue and WBC, but no changes in bone marrow. Different MiRNAs in different tissues and at different times.

• Development of hemoglobin adduct methods and data.
  – Vesper method set up.
    • Exogenous adducts not found in exposed rat blood
    • Endogenous adducts are found

• Endogenous vs Exogenous formyl-lysine.
  – Collaboration with MIT

• Development of DNA-Protein Cross-link analysis

• Rat and primate comparisons of DPC and adducts vs IRIS human estimates.

• Second primate study to thoroughly examine DNA adduct and DPC formation, epigenetic alterations, globin adducts and formyl-lysine.
MicroRNAs (miRNAs) are Important Epigenetic Regulators of Gene Expression

- Discovered in early 1990s
- Recognized as important biological regulators in early 2000s

miRNAs regulate gene expression in three ways:

1. Decay of target mRNA
2. Translational repression
3. Cleavage of newly translated polypeptides

(Filipowicz, 2008)
Nonhuman Primate Project

- Cynomolgus macaques were exposed to 0, 2, or 6 ppm $^{13}$CD$_2$ formaldehyde for 6 h/day for 2 days.
- RNA samples were collected from the maxilloturbinate and hybridized to miRNA microarrays to compare genome-wide miRNA expression profiles of formaldehyde-exposed versus unexposed samples.
- 13 MicroRNAs had altered expression.
- Inhibition of apoptosis genes was predicted and demonstrated (Rager et al., 2013, EHP).
Rodent Project Design

- Rats were exposed to 2 ppm $^{13}$CD$_2$ formaldehyde for 6 h/day for 28 days
- Time-matched control rats received clean air under the same conditions
- RNA samples were collected from the nose, circulating white blood cells, and bone marrow
- RNA samples were hybridized to the Agilent Rat miRNA Microarray to compare genome-wide miRNA expression profiles of formaldehyde-exposed versus unexposed samples

Genome-wide miRNA expression profiles were assessed throughout three regions: (1) nose, (2) circulating white blood cells, and (3) bone marrow
Formaldehyde as a source of N⁶-formyllysine

- Formaldehyde is relatively abundant: 10-100 µM in human plasma
- **Exogenous sources**: Environmental and occupational
- **Endogenous sources**: Demethylation of DNA, RNA and histones; biosynthesis of purines, thymidine and amino acids
Inhalation Exposure of Rats to $[^{13}\text{C}\text{D}_2]$-Formaldehyde leads to Formation of Labeled N$^6$-formyllysylsine in Nasal Tissue

Inhalation Exposure of Rats to $[^{13}\text{C}\text{D}_2]$-Formaldehyde leads to Formation of Labeled N$^6$-formyllysylsine in Nasal Tissue

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Inhalation Exposure of Rats to $[^{13}\text{C}\text{D}_2]$-Formaldehyde leads to Formation of Labeled N$^6$-formyllysylsine in Nasal Tissue

Inhalation Exposure of Rats to $[^{13}\text{C}\text{D}_2]$-Formaldehyde leads to Formation of Labeled N$^6$-formyllysylsine in Nasal Tissue
Endogenous and Exogenous N⁶-formyllysine Following a 6hr 9 ppm \([^{13}\text{CD}_2]-\text{Formaldehyde}\) Exposure

N⁶-Formylation per 10⁴ Lys

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Nasal Epithelium</th>
<th>Lung</th>
<th>Liver</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adduct type</td>
<td>Endo</td>
<td>Exog</td>
<td>Endo</td>
<td>Exog</td>
</tr>
<tr>
<td>Total Protein</td>
<td>2 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>3 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>2 ± 0.4</td>
<td>0.8 ± 0.1</td>
<td>4 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td>Membrane</td>
<td>2 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>3 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>Soluble nuclear</td>
<td>2 ± 1.0</td>
<td>0.5 ± 0.2</td>
<td>4 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>Chromatin bound</td>
<td>2 ± 0.4</td>
<td>0.2 ± 0.01</td>
<td>3 ± 0.2</td>
<td>ND</td>
</tr>
</tbody>
</table>
Formaldehyde Globin Adducts

- The method of imidazolidone formation of formaldehyde on N-terminal valine and the adjacent amino acid adapted from Ospina et al.
- Incubation of washed RBC or isolated globin with $[^{13}\text{CD}_2]$-formaldehyde resulted in exogenous adduct formation.
- The limit of detection (LOD) was 0.025 pmoles on column.
- No exogenous Hb-FA adducts were detected in rat globin following 1 day nor 5 day exposures to 10 ppm formaldehyde (6 hr/day).
- Endogenous levels were >500x above the LOD.
- We conclude that inhaled formaldehyde does not get to circulating blood.
Conclusions

• We have developed a series of highly specific and ultrasensitive methods that comprehensively demonstrate that inhaled formaldehyde does not reach distant tissues of rats and nonhuman primates.

• These methods utilize $[^{13}\text{C}]_2$-formaldehyde for the exposures so that both endogenous and exogenous DNA, globin and formyl-lysine adducts can be distinguished and quantitated.

• The assays were conducted in two independent laboratories and have confirmed that $[^{13}\text{C}]_2$-formaldehyde does not reach distant tissues such as blood and bone marrow.

• This research raises serious issues regarding the plausibility that inhaled formaldehyde causes leukemia. It seriously challenges the epidemiologic studies in several ways, including accurate exposure assessment, confounders and a lack of consistency across human and animal evaluations of carcinogenesis.
Future Studies and Questions

- Human CD 34+ cells to establish endogenous adduct amounts.
- Human bone marrow to compare with monkey data.
- Human nasal turbinates to establish endogenous adduct amounts.
- A primate study to examine additional tissues and WBC from monkeys exposed to $^{13}$CD$_2$-formaldehyde for epigenetic changes in MicroRNA, formyl-lysine.
- This new primate study will also provide high quality tissues for DNA adducts and DNA-protein cross-links.
- What are the relationships between DPC and DNA adducts?
Biomarkers of Formaldehyde Exposure: DPC vs. Adducts

DNA Lesions at 6 ppm formaldehyde normalized by time of exposure

DPC Study: 6 ppm $^{14}$C-formaldehyde for 6 hours
Adduct Study: 6 ppm $^{13}$CD$_2$-formaldehyde for 6 hours for 2 days

Question: What data supports the IRIS statement that humans are exposed to more than twice as much formaldehyde as rats?

DPC Data: Heck et al (1990) Toxicology
HPLC-MS/MS analysis of endogenous and exogenous dG-CH$_2$-Cys

Endogenous crosslink
dG-CH$_2$-Cys

Exogenous crosslink
dG-$^{13}$CD$_2$-Cys

Endogenous dG-CH$_2$-Cys can be detected in rat liver
Tryptic digestion of AGT-CH$_2$-dG Crosslink

+ 3 $m/z$ increase

$m/z$: 532.2804$^{(+3H)}$
MW: 1593.84 Da

$m/z$: 533.2950$^{(+3H)}$
MW: 1596.88 Da

Tryptic Cleavage Sites

AARAVGGAMRGNPPVILIP-Cys-HRVV

30 min digestion with immobilized trypsin

$^{13}$CD$_2$O:CH$_2$O incubation

QTOF: 532.0-534.0 $m/z$
Signal: 140 counts

12-mer AGT peptide

FA derived crosslink

24mer AGT-dG crosslink digested with trypsin to 12mer crosslink
AGT-CH$_2$-nucleotide and DNA crosslinks

**Reaction and sample preparation**

$T_7$GT$_7$ (or calf thymus DNA) + 12-mer AGT + formaldehyde $\rightarrow$ 37 $^\circ$C, pH 7.0, 23 h

AGT- $T_7$GT$_7$ (or DNA) crosslink $\rightarrow$ DNA digestion

AGT- dG crosslink $\rightarrow$ peptides digestion

dG-CH$_2$-Cys $\rightarrow$ HPLC-MS/MS

**Complete digestion of AGT-CH$_2$-nucleotide to dG-CH$_2$-Cys**

**Complete digestion of AGT-CH$_2$-DNA to dG-CH$_2$-Cys**
The Saga of Four Known Human Carcinogens

• Vinyl chloride, formaldehyde, acetaldehyde and ethylene oxide cause cancer in humans and experimental animals.

• All four of these chemicals are genotoxic and form DNA adducts.

• Identical endogenous DNA adducts are also formed in every living cell.

• The relationships between endogenous and exogenous DNA adducts and the induction of mutations and cancer are being investigated.
The Exposome

• Chris Wild proposed that we should be considering the “Exposome” for cancer etiology. Wild, C: CEBP 14: 1847-1850, 2005
  – Under this view, the assessment of exposures should not be restricted to chemicals entering the body from air, water, food, smoking, etc., but should also include internally generated toxicants produced by the gut flora, inflammation, oxidative stress, lipid peroxidation, infections, and other natural biological processes. In other words, we must focus upon the ‘internal chemical environment’ arising from all exposures to bioactive chemicals inside the body

• More recently, Martyn Smith et. al. made similar statements. Smith, M: Chemico Biological Interactions 192: 155-159, 2011
  – The question arises as to how to find the causes of the majority of de novo AMLs that remain unexplained. We propose that we should attempt to characterize the 'exposome' of human leukemia by using unbiased laboratory-based methods to find the unknown 'environmental' factors that contribute to leukemia etiology.
### Steady-state Amounts of Endogenous DNA Damage

<table>
<thead>
<tr>
<th>Endogenous DNA Lesions</th>
<th>Number per Cell</th>
<th>Endogenous DNA Lesions</th>
<th>Number per Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abasic sites</td>
<td>50,000</td>
<td>AcrdG</td>
<td>120</td>
</tr>
<tr>
<td>OHEtG</td>
<td>3,000</td>
<td>M₁dG</td>
<td>60</td>
</tr>
<tr>
<td>7-(2-Oxoethyl)G</td>
<td>3,000</td>
<td>N²,3-Ethenoguanine</td>
<td>36</td>
</tr>
<tr>
<td>8-oxodG</td>
<td>2,400</td>
<td>1N²-Etheno dG</td>
<td>30</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>1,000-4,000</td>
<td>1N⁶-Etheno dA</td>
<td>12</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1,000-5,000</td>
<td>O⁶-Methyl dG</td>
<td>2</td>
</tr>
<tr>
<td>7-Methylguanine</td>
<td>2,370</td>
<td>Total</td>
<td>60,000 +</td>
</tr>
</tbody>
</table>
Mutations Are Biomarkers of Effect, but They Do Not Go Through Zero

• In contrast to most DNA adducts, mutations do not go through zero.

• Rather, they reach a background level that reflects the summation of mutations arising from endogenous DNA damage and repair that occurs in cells.

• The dose-response may be linear or nonlinear.

• There may be an inflection point for a dose response curve where the number of mutations increases nonlinearly above the spontaneous level, or there may be a linear increase with data points that are not significantly different from controls at lower doses.

• The point at which the mutations increase is where the exogenous DNA damage starts driving the biology that results in additional mutations.
Linearized Multistage Model for Cancer Risk Assessment

• The LMS model has been the default model for the EPA since 1986.

• It is highly public health conservative.

• Dr. Kenny Crump, the originator of the LMS model, has stated that this model
  – incorporates no biology, and
  – will over estimate cancer risks by several orders of magnitude if nonlinear data are known
Default

• The word default first came into use in the 1200’s.
  – A failure to meet one’s obligation
  – A sin

• The above concept is certainly applicable to risk assessment.
  – We have failed to meet our obligation to use the best science when we resort to defaults.
Collaborators and Sponsors

- Kun Lu
- Ben Moeller
- Genna Kingon
- Rui Yu
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- Texas Commission for Environmental Quality
- NIEHS Superfund Basic Research Program (P42-ES 5948)
- NIEHS Center for Environmental Health and Susceptibility (P30 ES 10126)
Historical Control Data for HPRT and TK Mutations in vitro

Penman and Crespi, Environ Mol Mut 10:35-60, 1987
Repair of Formaldehyde DNA Lesions

Aged Aldh2−/− Fancd2−/− mice succumb to bone marrow failure.

Acute leukaemia in \textit{Aldh2}^{-/-} \textit{Fancd2}^{-/-} mice.

The FA Core Genes are Synthetically Lethal to DT40 Cells: but the effects of endogenous HCHO can be reversed by 2-mercaptetanol