GENOTOXIC AND NON-GENOTOXIC MECHANISMS FOR ACRYLAMIDE CARCINOGENICITY

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OUTLINE

• History
• Summaries of evidence for:
  genotoxicity, pre- and post-2005;
  endocrine disruption, post-2005;
  *in vitro* effects post-2005.
• Carcinogenic mechanism for acrylamide
• Chronic NCTR/NTP cancer bioassays
• PBPK modeling & extrapolation to dietary risks
• Conclusions & remaining issues
HISTORY OF ACRYLAMIDE RESEARCH

• Occupational exposures – peripheral neuropathy
• Discovery of ppm levels of AA in many baked and fried starchy foods (2002 M. Törnqvist et al./Swedish Food Authority)
• Two 2 y carcinogenicity bioassays for AA in F344 rats
• Short-term studies in mice
• 2003 FDA priority nomination to NTP – mechanistic studies and chronic bioassays (cancer - AA and GA in mice and rats; neurotoxicity - rats)
Evidence for GA as the Genotoxic Metabolite of AA (pre-2005)

- Structural similarity to ethylene oxide, glycidol
- GA reactivity with DNA bases >> AA
- GA-DNA adducts (N7-Gua & N3-Ade) in all tissues tested
- DNA adducts accumulate with repeated dosing
- GA more mutagenic than AA in vitro (Salmonella, Big Blue mouse embryonic fibroblasts - cll mutations predominately G:C → T:A transversions
- Overlapping F344 rat tumor sites from ethylene oxide, glycidol, acrylonitrile & AA (CNS, peri-testicular mesothelium, thyroid, mammary)
- N-Methylolacrylamide carcinogenic in B6C3F₁ mice (not F344 rats)
- CNS tumors only from DNA-damaging agents
Evidence for GA as the Genotoxic Metabolite of AA in vivo (2005-present)

- GA causes DNA adducts, micronucleus & DNA damage (comet assay), germ cell mutations & dominant lethality in wt, but not CYP2E1 ko mice (2005)
- AA is a genotoxic mutagen for adult Big Blue mice via metabolism to GA (↑ MF at Hprt (spleen) & cll (liver) – major mutation G:C → T:A transversions) (2006)
- GA is a genotoxic mutagen (↑ MF at Tk and Hprt - spleen) in neonatal Tk+/− mice (2008)
- GA is a genotoxic carcinogen in liver from neonatal mice (2008)
### Proposed Endocrine Mechanisms for AA Carcinogenesis in F344 Rats

<table>
<thead>
<tr>
<th>Type of Cancer &amp; Gender Affected</th>
<th>Hormonal Aberration Contributing to Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid follicular adenoma/adenocarcinoma (M/F)</td>
<td>Prolonged TSH stimulus DA dysregulation</td>
</tr>
<tr>
<td>Mesothelioma – tunica vaginalis (scrotum)</td>
<td>Prolonged stimulation by PRL, LH, FSH DA dysregulation</td>
</tr>
<tr>
<td>Fibroadenoma/carcinoma mammary gland (F)*</td>
<td>Prolonged stimulation by progesterone, PRL (F) DA dysregulation</td>
</tr>
<tr>
<td>CNS tumors (M/F)</td>
<td>Not Discussed</td>
</tr>
</tbody>
</table>

[Shipp et al., *Crit. Rev. Toxicol.* 36, 481 (2006)]
The Effects of Sub-chronic Acrylamide Exposure on Gene Expression, Neurochemistry, Hormones, and Histopathology in the Hypothalamus-pituitary-thyroid Axis of Male Fischer 344 Rats

NEUROENDOCRINE MECHANISMS FOR AA CARCINOGENICITY IN F344 RATS

- AA in drinking water: 0, 2.5, 10, 50 mg/kg in male F344 rats
- 2.5 mg/kg = cancer bioassay dose; 10 mg/kg intermediate; 50 mg/kg neurotoxic but not lethal
- 14 day exposure (minimize accommodation)
- Eliminate estrus cycle considerations (males only)
- Brain neurotransmitters (dopamine & 5HT) and metabolites (LC/MS/MS)
- Serum PRL, FSH, LH, TSH, T3/T4, sex steroids
- Genomic Analysis-Macroarrays (ca. 2,400 cDNA probes): expression of hormone releasing factors; neurotransmitter receptors in hypothalamus, striatum, pituitary; thyroid; oxidative stress; apoptosis; cell proliferation
- Histology – thyroid, pituitary, brain, testes; cell proliferation
Hormonal Mechanisms for AA Carcinogenesis - Summary

- F344 rat -14 d study: $< 50 \text{ mg/kg bw/d} \Rightarrow$ steady state $[\text{AA, GA}] < 25 \, \mu\text{M}$
- Some evidence for hormonal changes from direct effects on thyroid and testes
- No evidence for mRNA changes in HPT-related genes; oxidative stress; etc.
- No evidence for increased cell proliferation in target tissues of male F344 rat
- No evidence for disruption of HPT axis by demonstrably toxic doses of AA
Effects of AA *in vitro* (2005-present)

- Oxidative stress through GSH depletion only at [AA, GA] ~ mM levels (2005-7)
- Effects on kinesin-related microtubular proteins (mitotic spindle) at [AA, GA] ≥ 100 μM (2007)
- Effects on human lymphocytes – comet assay at [AA] ≥ 0.5 μM (2006)
- mRNA expression – MCF7, CaCo-2 cells; minor changes in fold change ≤ 10 μM AA or GA (2007)
- Cancer bioassay doses
  F344 rat: ≤ 3 mg/kg bw/d => [AA, GA] ≤ 2 μM
Effects of Acrylamide *in vitro* - Summary

- No evidence for oxidative stress effects at AA concentrations relevant to cancer bioassay doses
- No evidence for effects on microtubular-associated proteins at [AA or GA] relevant to cancer bioassays (Neurotoxicity?)
- Consistent evidence for DNA damage in target tissues (comet assay) at [AA] relevant to cancer bioassay
- Minimal evidence for mRNA expression changes at [AA or GA] relevant to cancer bioassays
## CHRONIC CARCINOGENICITY STUDY
**B6C3F₁ MICE – WATER (F. Beland)**

<table>
<thead>
<tr>
<th>Test agent</th>
<th>Sex</th>
<th>Dosed water (mM)</th>
<th>Target daily intake (mg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA &amp; GA</td>
<td>Male &amp; female</td>
<td>0.70</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.175</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0875</td>
<td>1.75</td>
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<td>0</td>
<td>0</td>
</tr>
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<td>Test agent</td>
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</tr>
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<td>------------------------------</td>
</tr>
<tr>
<td>AA &amp; GA</td>
<td>Male &amp; female</td>
<td>0.70</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.35</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.175</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0875</td>
<td>0.625</td>
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<td>0</td>
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PRELIMINARY GROSS PATHOLOGY: LESIONS IN MALE B6C3F₁ MICE TREATED CHRONICALLY

Acrylamide

Glycidamide

<table>
<thead>
<tr>
<th>Compound</th>
<th>Harderian gland</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM</td>
<td>5 (0.83)</td>
<td>10 (1.67)</td>
<td>2 (0.33)</td>
</tr>
<tr>
<td>0.0875 mM</td>
<td>10 (1.67)</td>
<td>20 (3.33)</td>
<td>5 (0.83)</td>
</tr>
<tr>
<td>0.175 mM</td>
<td>15 (2.50)</td>
<td>30 (5.00)</td>
<td>10 (1.67)</td>
</tr>
<tr>
<td>0.35 mM</td>
<td>20 (3.33)</td>
<td>40 (6.67)</td>
<td>15 (2.50)</td>
</tr>
<tr>
<td>0.70 mM</td>
<td>25 (4.17)</td>
<td>50 (8.33)</td>
<td>20 (3.33)</td>
</tr>
</tbody>
</table>

Number of lesions

0 mM  0.0875 mM  0.175 mM  0.35 mM  0.70 mM
LESIONS IN MALE B6C3F\(_1\) MICE TREATED NEONATALLY

Liver tumor incidence (%)

- Acrylamide
- Glycidamide

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Tumor Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>1.0</td>
</tr>
<tr>
<td>0.14 mmol/kg</td>
<td>1.3</td>
</tr>
<tr>
<td>0.70 mmol/kg</td>
<td>1.0</td>
</tr>
<tr>
<td>3.5 mmol/kg</td>
<td>3.5</td>
</tr>
</tbody>
</table>
NCTR CARCINOGENICITY BIOASSAYS
AA & GA : PROJECTED SCHEDULE

• In-life complete 8-07
• Pathology complete 07-08
• PWG 01-09
• Tumor data available on NTP website
• Statistics & QA complete 03-09
• BSI report preparation 08-09
• NTP Technical Report Subcommittee 11-09
Carcinogenic Mechanism(s) for Acrylamide

• A compelling body of evidence for a DNA-reactive mechanism for AA carcinogenicity via metabolism to GA
• Reproducibly carcinogenic at multiple anatomic sites in both rats and mice
• Tumor sites in mice are indicative of small epoxide carcinogens
• The only agents known to induce tumors of the CNS and peritesticular mesothelium in rats are all DNA-reactive (mutagenic)
• The hormonal disruption & oxidative stress mechanisms proposed for AA as tissue-specific alternatives to a DNA-reactive mechanism are highly speculative, unsupported in vivo
• Other mechanisms possible at higher doses; likelihood decreases as level of exposure decreases => less relevant for risk assessment of human dietary exposures
PBPK/PD Model for Acrylamide and Its Metabolites In Mice, Rats, and Humans

J.F. Young, R.H. Luecke, D.R. Doerge
PREDICTION OF STEADY STATE HUMAN DNA ADDUCTS FROM DIETARY EXPOSURES USING PBPK/PD MODELING

- PBPK/PD (urinary metabolites & Hb adducts from non-smokers – 0.4 µg/kg bw/d)
  0.26 N7-GA-Gua/10^8 nucleotides

- Empirical relationship between rodent GA-Hb and DNA adducts (diet and 1 mg/kg bw/d)
  0.2-0.3 N7-GA-Gua/10^8 nucleotides

- Range of estimated human cancer risks from dietary AA using rat tumor data
  1 x 10^{-4} (F thyroid) to 4 x 10^{-4} (F mammary)
CONCLUSIONS

• Hypothesis: AA = genotoxic carcinogen via metabolism to GA
• Tumor incidences from rodent bioassays (“external” dose)
• GA-Hb adduct measurements in humans exposed through diet only (“internal” dose)
• PBPK predictions of steady state DNA adduct levels in human tissues (“effective” dose) - cancer risk assessment
What to do about a genotoxic carcinogen that is pervasive throughout the diet?
- Significant proportion of total caloric content of global agriculture in cereals and tubers
- Diet-Cancer linkage robust
- Sufficiently powered epidemiological studies to relate AA in diet with cancers at specific sites unlikely?
- What about exposure to other known cooking carcinogens? (B[a]P (4 ng/kg bw/d), HAAs (15), furan (300), AA (400-1000))
- Unknown compounds?
- Holistic risk assessment for all “cooking carcinogens”? 