Draft NTP Technical Report TR592 on Dietary Zinc (Feed Studies)

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Nomination

• Nominated by private individuals
  – Investigate the carcinogenicity of **zinc deficiency** based on data showing that deficiency of some vitamins and minerals can cause DNA damage

• Additional nomination by the Agency for Toxic Substances and Disease Registry (ATSDR)
  – Increasing population size exposed to **excess zinc** through dietary supplementation
  – Lack of studies examining the carcinogenicity of zinc
Background

- Zinc is an essential trace element with various critical biological functions
  - Major component in DNA-binding proteins that have catalytic or structural function

- Recommended Dietary Allowance (IOM, 2002)
  - Men and women: 8 to 11 mg
  - Pregnant and lactating women: 11 to 13 mg

- Zinc intake in many men (aged 18+) and women (aged 14+) is below the estimated average requirement (Data from NHANES 2007-2010)
Toxicology

- Altering the intake/concentration of zinc may alter the pharmacokinetics or toxicity/carcinogenicity of other metals
  - Interaction with cadmium (Cd)
    - Excess zinc prevents cadmium-induced carcinogenicity
    - Zinc deficiency enhances Cd-induced toxicity and carcinogenicity
  - High zinc levels interfere with absorption of iron and copper
Test Article and Dose Selection

• Zinc carbonate
  – Zinc salt recommended by the American Institute of Nutrition for the AIN-93M rodent diet
  – Zinc content was determined to be 56.6%*

• Dose Selection
  – Based on critical role of zinc in maintenance of life

* Levine et al (in press) Characterization of Zinc Carbonate Basic as a Source of Zinc in a Rodent Study Investigating the Effects of Dietary Deficiency or Excess
Study Design Considerations

• Management of zinc levels
  – AIN-93M synthetic diet, low-zinc diet with known quantities of zinc added to achieve appropriate zinc levels
  – Elimination of extraneous sources of zinc throughout the study (e.g., metal cages, feeders, drinking water)
  – Avoidance of zinc contamination (e.g., sample collections)
NTP Program of Study for Dietary Zinc

- **2-Year** feed studies in Harlan Sprague Dawley rats
  - 3, 6, 9, and 12-month interim time points for blood level measurements of zinc, copper, and iron

- **Genotoxicity** testing
  - 12-month interim time point for Comet assay in colon and blood
  - *In vivo* micronucleus
Genetic Toxicity Results for Dietary Zinc in Rats

• *In vivo* blood micronucleus
  – Negative in males and females for both diets (deficient or excess)

• DNA damage (Comet Assay)

<table>
<thead>
<tr>
<th></th>
<th>Zinc deficient</th>
<th>Zinc excess</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male rats</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>Positive at 12 months</td>
<td>Positive at 12 months</td>
</tr>
<tr>
<td>Colon cells</td>
<td>Negative</td>
<td>Increase in DNA migration</td>
</tr>
</tbody>
</table>

| **Female rats**    |                                     |                                          |
| Leukocytes         | Positive at 9 and 12 months         | Negative                                 |
| Colon cells        | Decrease in DNA migration           | Increase in DNA migration                |
Chronic Rat Studies

Dietary Zinc
HSD: Sprague Dawley SD Rats – Survival

No treatment-related effects on survival.
HSD: Sprague Dawley SD Rats – Body weights

Male Rats

Female Rats

Mean body weight in grams

No treatment-related effects on body weight.
Zinc and Copper Blood Concentrations

- **Diet containing Deficient Zinc**
  - No changes in blood levels of zinc or copper at any time point

- **Diet containing Excess Zinc**
  - Males and females at Day 19 (250 and 500 ppm)
    - Increase in Zinc (23-43%)
    - Decrease in Copper (50-75%)
  - Return to homeostasis for subsequent time points
  - Transient decrease of blood iron levels in females receiving diet with 500 ppm zinc (3-6 months)
## Nonneoplastic Lesions in the Pancreas of Females

### Number of animals with lesion (average severity grade of lesion: 1=minimal, 2=mild, 3=moderate, 4=marked)

* P < 0.05 by Poly-3 test

<table>
<thead>
<tr>
<th></th>
<th>Zinc Deficient</th>
<th>Excess Zinc</th>
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<tr>
<td></td>
<td>3.5 ppm</td>
<td>7.0 ppm</td>
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<tr>
<td>Number Examined Microscopically</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Acinus, Atrophy</td>
<td>4 (1.0)</td>
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Number of animals with lesion (average severity grade of lesion: 1=minimal, 2=mild, 3=moderate, 4=marked)

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<th>38 ppm (Control)</th>
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<tbody>
<tr>
<td>Acinus, Adenoma, Multiple</td>
<td>13/50*</td>
<td>10/48</td>
<td>5/49</td>
<td>8/48</td>
<td>4/48</td>
</tr>
<tr>
<td>Acinus, Adenoma (includes multiple)</td>
<td>21/50</td>
<td>19/48</td>
<td>11/49</td>
<td>13/48</td>
<td>10/48</td>
</tr>
<tr>
<td>Acinus, Carcinoma</td>
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<td>1/49</td>
<td>0/48</td>
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- Higher incidences of acinar cell hyperplasia in deficient diet
- Higher incidences of adenoma, with statistically significant increased incidences of multiple adenomas in deficient diet
## Nonneoplastic Lesions in the Testis of Male Rats

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<th>38 ppm (Control)</th>
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<tr>
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<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Bilateral, Germinal Epithelium, Atrophy</td>
<td>7* (2.4)</td>
<td>1 (3.0)</td>
<td>0</td>
<td>0</td>
<td>1 (3.0)</td>
</tr>
<tr>
<td>Germinal Epithelium, Atrophy</td>
<td>3 (2.3)</td>
<td>0</td>
<td>5 (2.0)</td>
<td>3 (2.7)</td>
<td>4 (2.8)</td>
</tr>
<tr>
<td>Germinal Epithelium, Atrophy (Includes Bilateral)</td>
<td>10 (2.4)</td>
<td>1 (3.0)</td>
<td>5 (2.0)</td>
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Number of animals with lesion (average severity grade of lesion: 1=minimal, 2=mild, 3=moderate, 4=marked)

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Evidence of Carcinogenic Activity in Rats

• Diet deficient in zinc (3.5 or 7.0 ppm)
  – *Equivocal* in male Harlan Sprague Dawley rats administered diets deficient in zinc based on higher incidences of acinar adenomas of the pancreas and increased incidences of animals with multiple adenomas
  – *No Evidence* in female Harlan Sprague Dawley rats administered diets deficient in zinc

• There was an increased incidence of acinar atrophy of the pancreas in male and female rats
Evidence of Carcinogenic Activity in Rats

- **Diet containing excess zinc (250 or 500 ppm)**
  - *No Evidence* in male Harlan Sprague Dawley rats administered excess zinc diets
  - *No Evidence* in female Harlan Sprague Dawley rats administered excess zinc diets

- There was an increased incidence of bilateral atrophy of the germinal epithelium in the testes of male rats