

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

Michael Wyde, Ph.D., DABT

Natasha Catlin, Ph.D.

Amy Brix, DVM, Ph.D.

Division of National Toxicology Program
National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
July 13, 2017



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

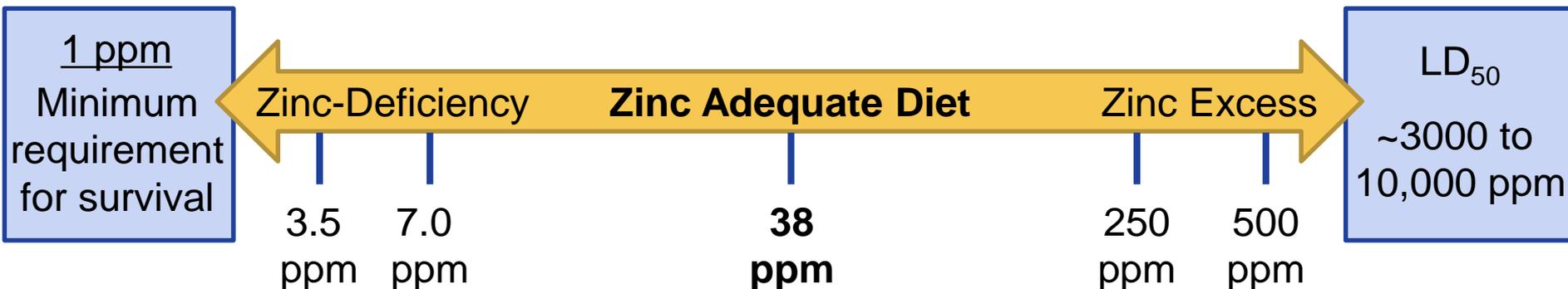
- Zinc is an essential trace element with various critical biological functions
 - Major component in DNA-binding proteins that have catalytic or structural function
- Recommended Dietary Allowance (IOM, 2002)
 - Men and women: 8 to 11 mg
 - Pregnant and lactating women: 11 to 13 mg
- Zinc intake in 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)**

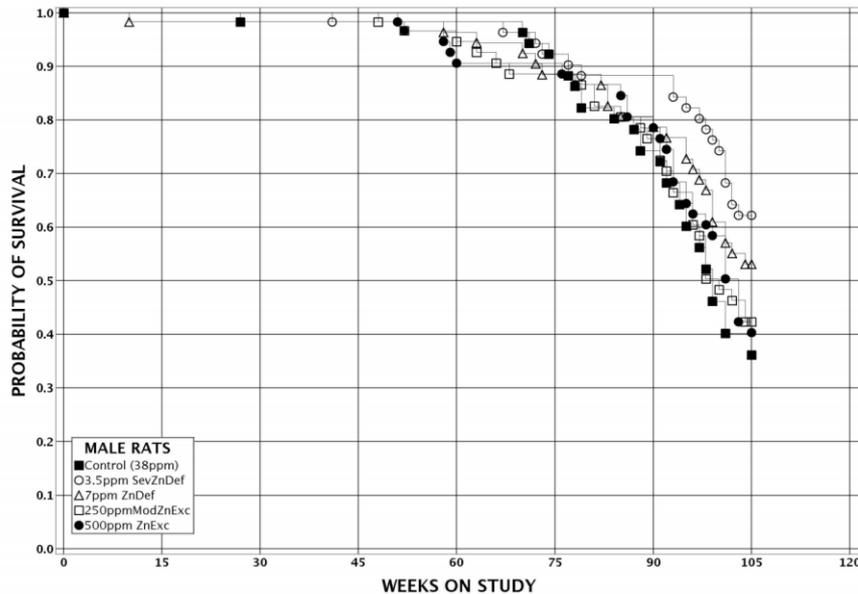
| | Zinc deficient | Zinc excess |
|--------------------|-----------------------------|---------------------------|
| Male rats | | |
| Leukocytes | Positive at 12 months | Positive at 12 months |
| Colon cells | Negative | Increase in DNA migration |
| Female rats | | |
| Leukocytes | Positive at 9 and 12 months | Negative |
| Colon cells | Decrease in DNA migration | Increase in DNA migration |

Chronic Rat Studies

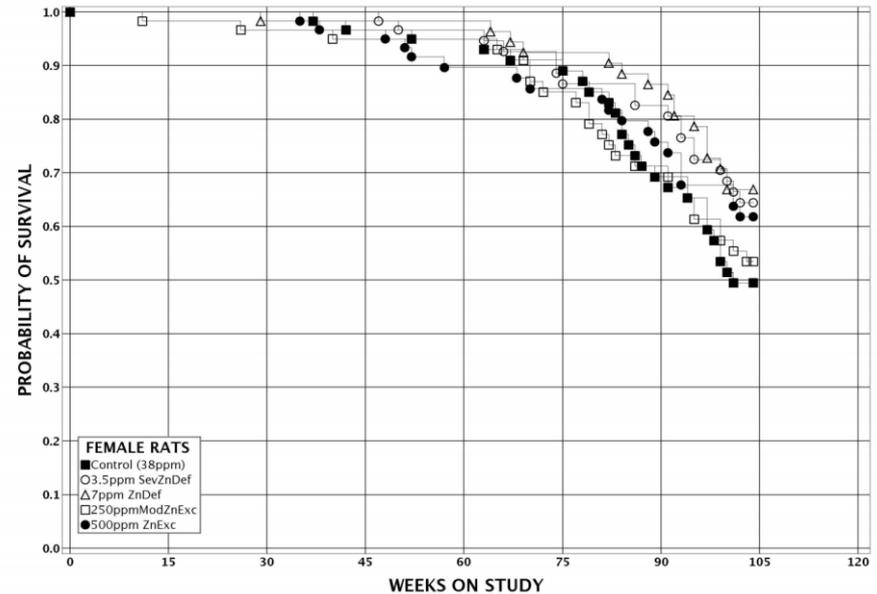
Dietary Zinc

HSD:Sprague Dawley SD Rats – Survival

Male Rats



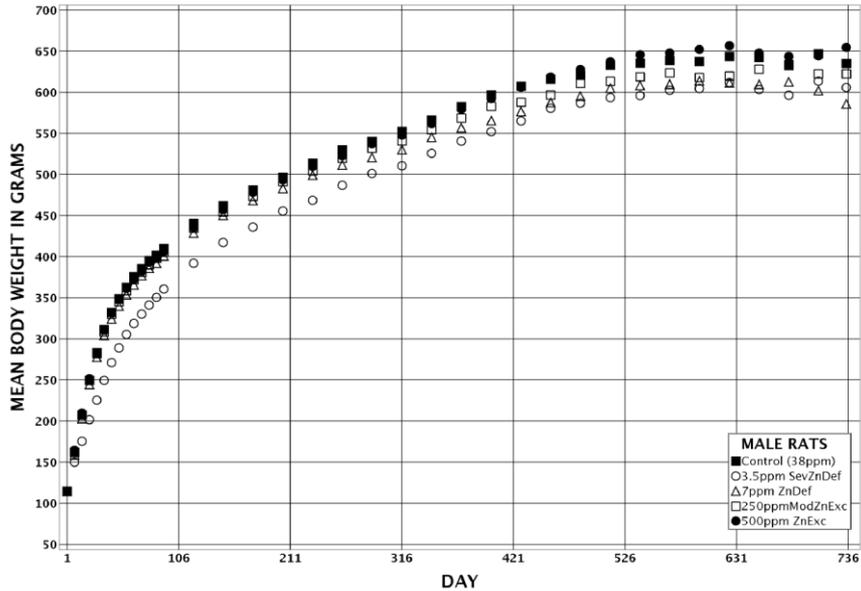
Female Rats



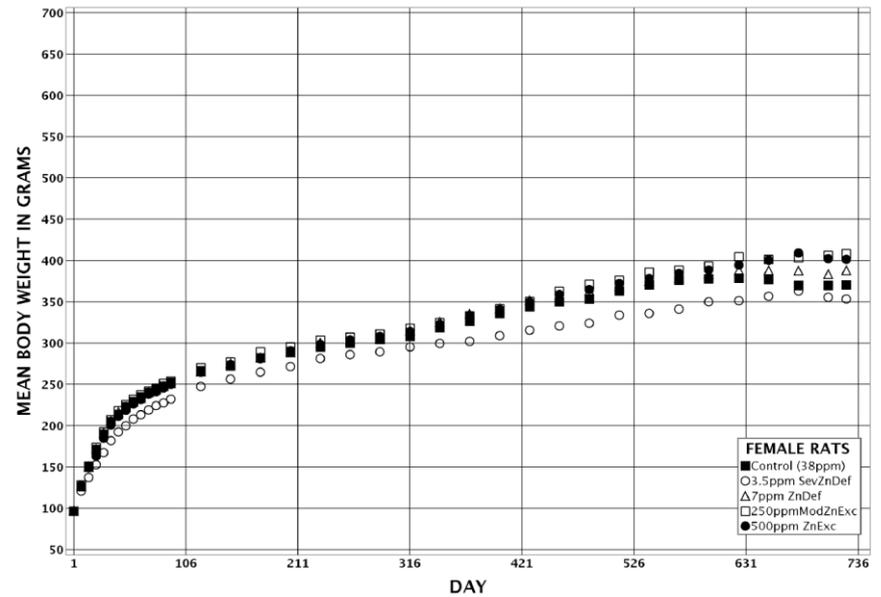
No treatment-related effects on survival.

HSD:Sprague Dawley SD Rats – Body weights

Male Rats



Female Rats



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

| | <u>Zinc Deficient</u> | | | <u>Excess Zinc</u> | |
|---------------------------------|-----------------------|---------|-----------------------------|--------------------|------------------|
| | 3.5 ppm | 7.0 ppm | 38 ppm (Control) | 250 ppm | 500 ppm |
| Number Examined Microscopically | 48 | 49 | 50 | 49 | 49 |
| Acinus, Atrophy | 4 (1.0) | 2 (1.0) | 2 (1.5) | 5 (1.2) | 10* (1.4) |

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

Incidences of Lesions in the Pancreas of Males

| | 3.5 ppm | 7.0 ppm | 38 ppm (Control) | 250 ppm | 500 ppm |
|-------------------------------------|---------|---------|---------------------|---------|---------|
| Acinus, Atrophy | 3/50 | 4/48 | 3/49 | 3/48 | 13/48** |
| Acinus, Hyperplasia | 32/50 | 23/48 | 23/49 | 21/48 | 28/48 |
| Acinus, Adenoma, Multiple | 13/50* | 10/48 | 5/49 | 8/48 | 4/48 |
| Acinus, Adenoma (includes multiple) | 21/50 | 19/48 | 11/49 | 13/48 | 10/48 |
| Acinus, Carcinoma | 0/50 | 0/48 | 1/49 | 0/48 | 0/48 |
| Acinus, Adenoma or Carcinoma | 21/50 | 19/48 | 11/49 | 13/48 | 10/48 |

* P < 0.05; ** P < 0.01 by Poly-3 test

Incidences of Lesions in the Pancreas of Males

| | 3.5 ppm | 7.0 ppm | 38 ppm (Control) | 250 ppm | 500 ppm |
|-------------------------------------|---------|---------|---------------------|---------|---------|
| Acinus, Atrophy | 3/50 | 4/48 | 3/49 | 3/48 | 13/48** |
| Acinus, Hyperplasia | 32/50 | 23/48 | 23/49 | 21/48 | 28/48 |
| Acinus, Adenoma, Multiple | 13/50* | 10/48 | 5/49 | 8/48 | 4/48 |
| Acinus, Adenoma (includes multiple) | 21/50 | 19/48 | 11/49 | 13/48 | 10/48 |
| Acinus, Carcinoma | 0/50 | 0/48 | 1/49 | 0/48 | 0/48 |
| Acinus, Adenoma or Carcinoma | 21/50 | 19/48 | 11/49 | 13/48 | 10/48 |

* P < 0.05; ** P < 0.01 by Poly-3 test

- 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

| | 3.5 ppm | 7.0 ppm | 38 ppm (Control) | 250 ppm | 500 ppm |
|--|----------|---------|---------------------|---------|---------|
| Number Examined Microscopically | 50 | 50 | 50 | 50 | 50 |
| Bilateral, Germinal Epithelium, Atrophy | 7* (2.4) | 1 (3.0) | 0 | 0 | 1 (3.0) |
| Germinal Epithelium, Atrophy | 3 (2.3) | 0 | 5 (2.0) | 3 (2.7) | 4 (2.8) |
| Germinal Epithelium, Atrophy (Includes Bilateral) | 10 (2.4) | 1 (3.0) | 5 (2.0) | 3 (2.7) | 5 (2.8) |

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

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