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

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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

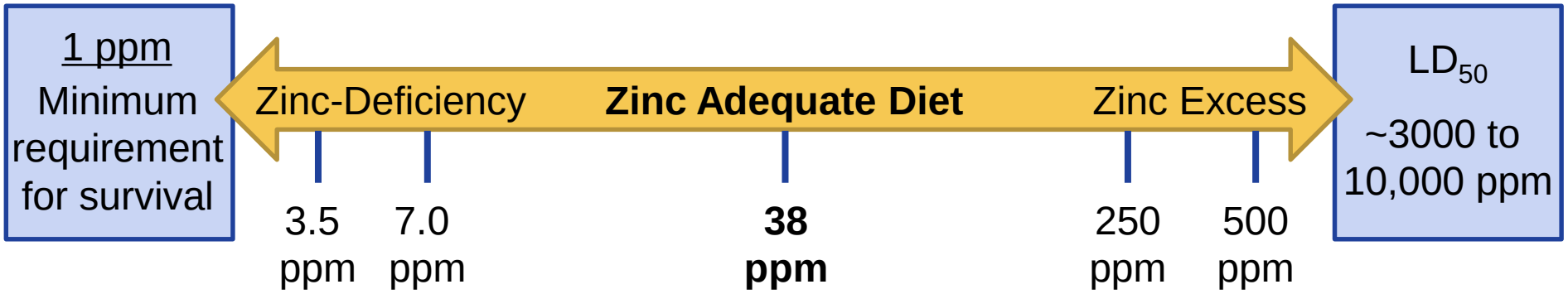
- Zinc is an essential trace element with various critical biological functions
  - Major component in DNA-binding proteins that have catalytic or structural function
- Recommended Dietary Allowance (IOM, 2002)
  - Men and women: 8 to 11 mg
  - Pregnant and lactating women: 11 to 13 mg
- Zinc intake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

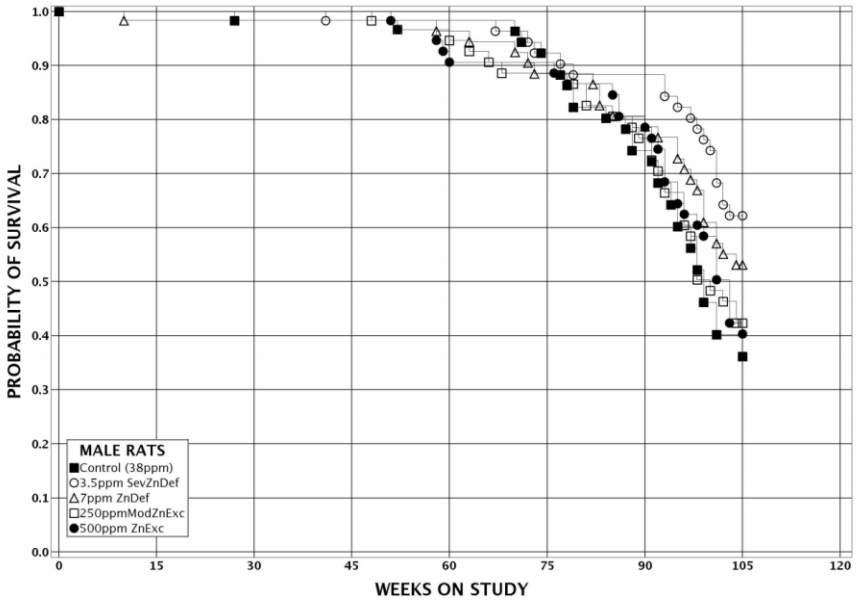
	Zinc deficient	Zinc excess
Male rats		
Leukocytes	Positive at 12 months	Positive at 12 months
Colon cells	Negative	Increase in DNA migration
Female rats		
Leukocytes	Positive at 9 and 12 months	Negative
Colon cells	Decrease in DNA migration	Increase in DNA migration

Chronic Rat Studies

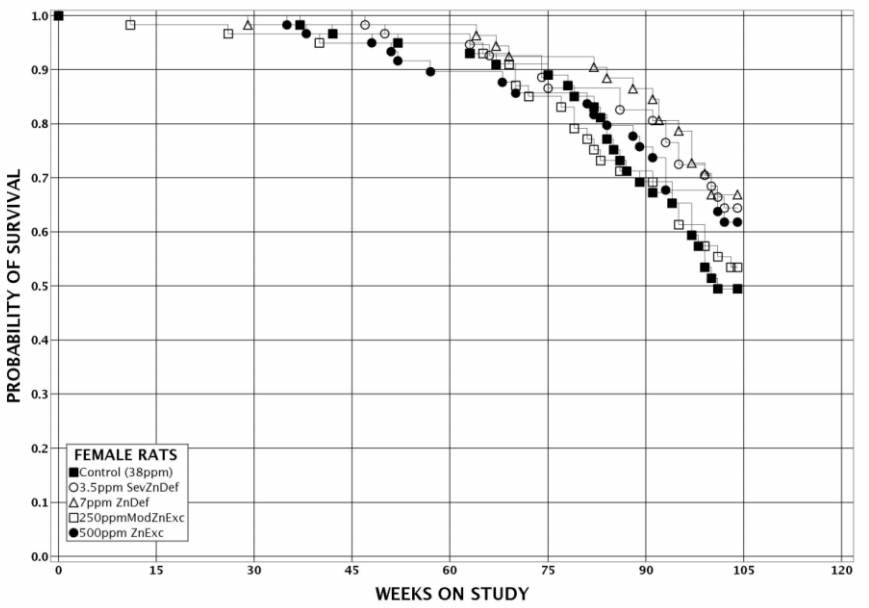
Dietary Zinc

HSD:Sprague Dawley SD Rats – Survival

Male Rats



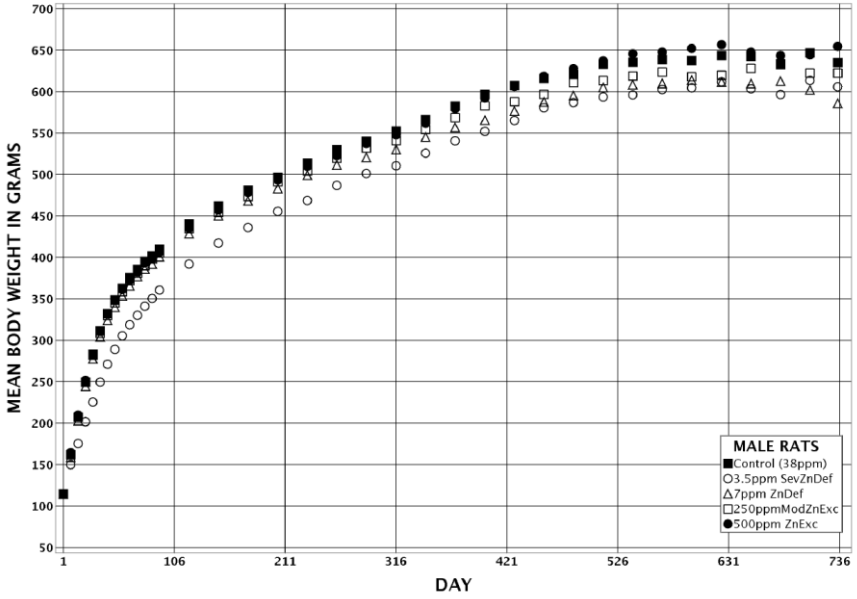
Female Rats



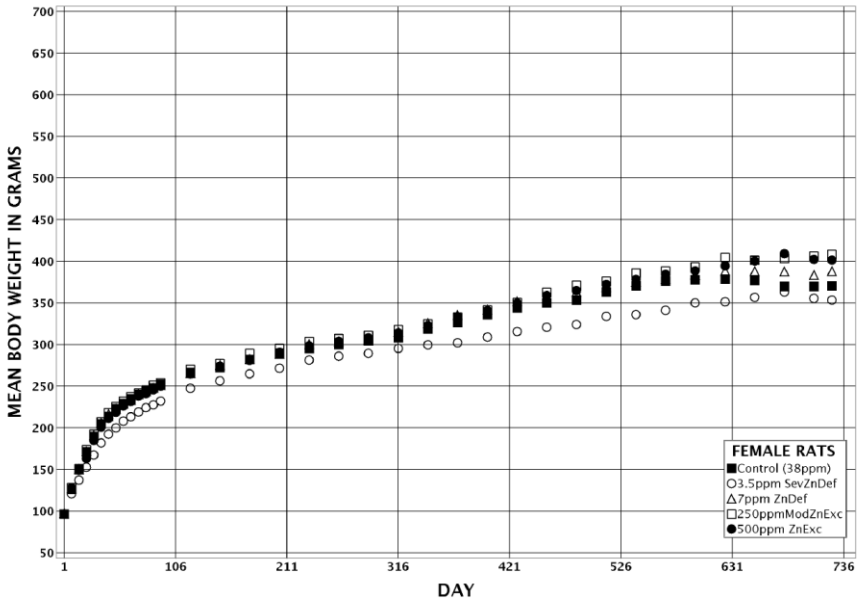
No treatment-related effects on survival.

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

	Zinc Deficient			Excess Zinc	
	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

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* $P < 0.05$; ** $P < 0.01$ 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