AN EVALUATION OF THE TOXICOLOGICAL EFFECTS OF DISCRETE SIZES OF SILVER NANOSCALE PARTICLES (AgNP) IN THE SPRAGUE DAWLEY RAT

Mary D. Boudreau
Division of Biochemical Toxicology
National Center for Toxicological Research (NCTR),
U.S. Food and Drug Administration,
Jefferson, AR, USA

National Center for Toxicological Research (NCTR)

- Conducts fundamental and applied peer-reviewed research designed to define the biological mechanisms of action underlying the toxicity of products regulated by the FDA
- Characterizes the toxicities and risks associated with exposure to specific chemicals of interest to the FDA
- Develops new scientific tools for the FDA to improve public health

FDA Nomination of AgNP to the National Toxicology Program (NTP)

- Rationale:
  - Increased widespread use in drugs, food, and cosmetic products
  - Lack of toxicology and pharmacokinetic data
  - Size may influence biological response.
- General scope of studies:
  - Conduct absorption, distribution, metabolism, and elimination (ADME) studies in rodents
  - Conduct subchronic dose-response toxicity studies in rodents
  - Include at least 2 sizes of well-characterized AgNP

Test Articles

Chemical Form	Morphology	Nominal Size
Citrate stabilized (OECD*) mono-dispersed AgNP	Spheres	~ 10 nm
Citrate stabilized (OECD) mono-dispersed AgNP	Spheres	~ 75 nm
Citrate stabilized (OECD) mono-dispersed AgNP	Spheres	~ 110 nm
Silver acetate	Ionic	Bulk

* Organization for Economic Co-operation and Development

Test Article Analysis by ICP-MS and Dynamic Light Scattering (DLS)

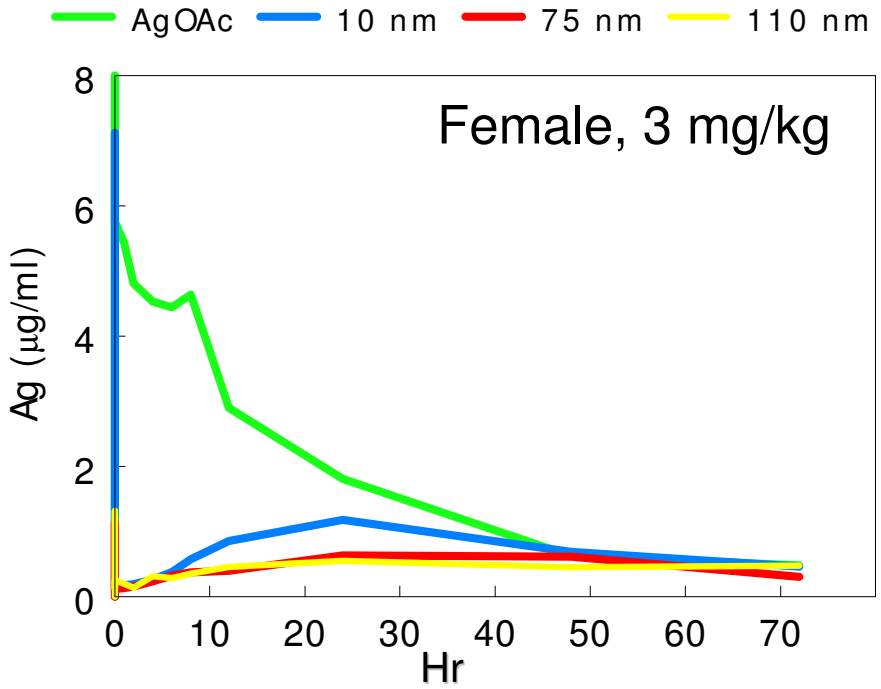
Sample	Nominal Ag conc. (mg/ml)	Actual Ag mass conc. (mg/ml)	Nominal size (nm)	PDI	Mean particle size (nm)	Intensity (%)	Z-average
Gold Std			60	0.105	63.5	100	56.4
10 nm AgNP	1.0	0.98	11	0.159	16.9	100	13.9
75 nm AgNP	1.0	0.98	75	0.170	75.4	100	62.4
100 nm AgNP	1.0	0.94	107	0.087	110.9	100	99.49
AgOAc	6.46	6.79	-	-	-	-	-

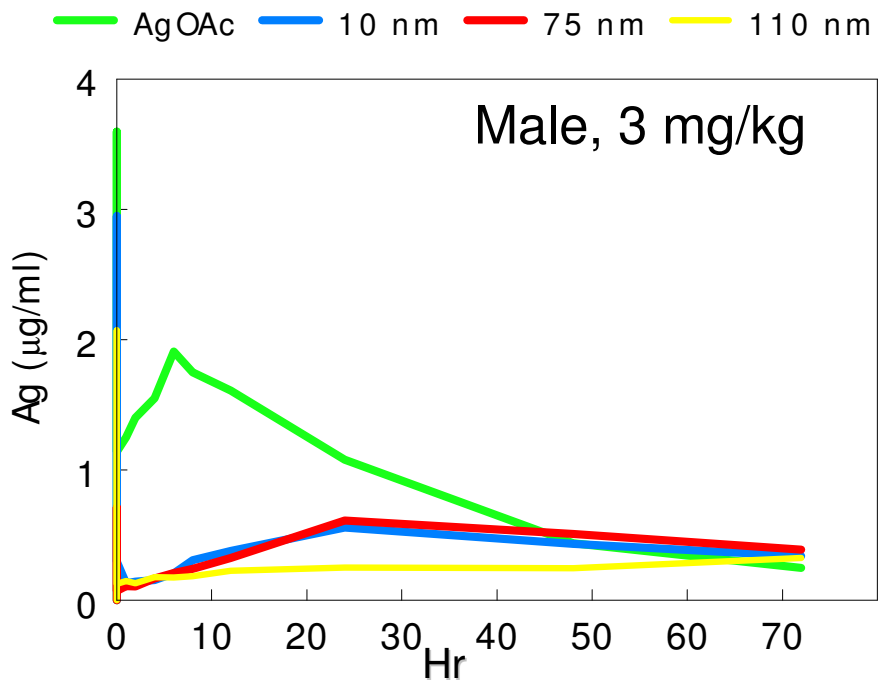
Acute Dose Study Design

- Pharmacokinetic Study
  - AgNP (10, 75, and 110 nm) and AgOAc
  - Single oral administration (10 mg/kg bw) or intravenous administration (3 mg/kg bw)
  - Sprague Dawley rats, 4 per sex per treatment
  - Blood collected at 13 time points over 72 h
  - Blood silver by mass evaluated by ICP-MS
- ADME Study
  - Single oral administration (20 mg/kg bw)
  - Sprague Dawley rats, 4 per sex per treatment
  - 24-h urine and feces collected each of 7 days
  - Tissues and organs collected for ICP-MS and TEM

Blood Levels of Silver Following Intravenous AgNP Administration

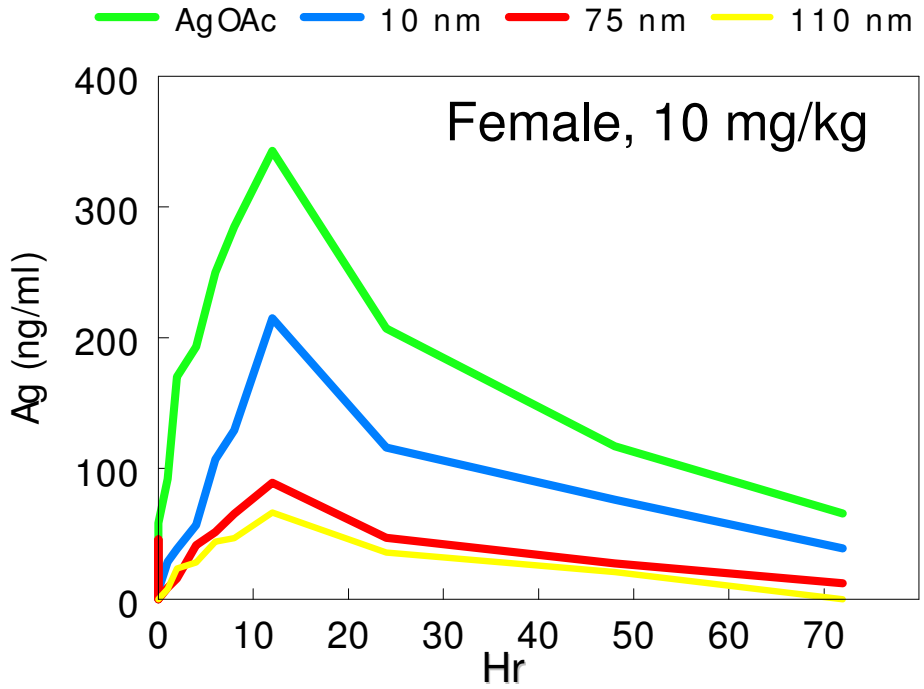
- The decrease in blood silver concentrations was followed by an increase in flux and a subsequent plateau that remained for at least 72 h
- Protein binding (albumin) by Ag may prevent clearance

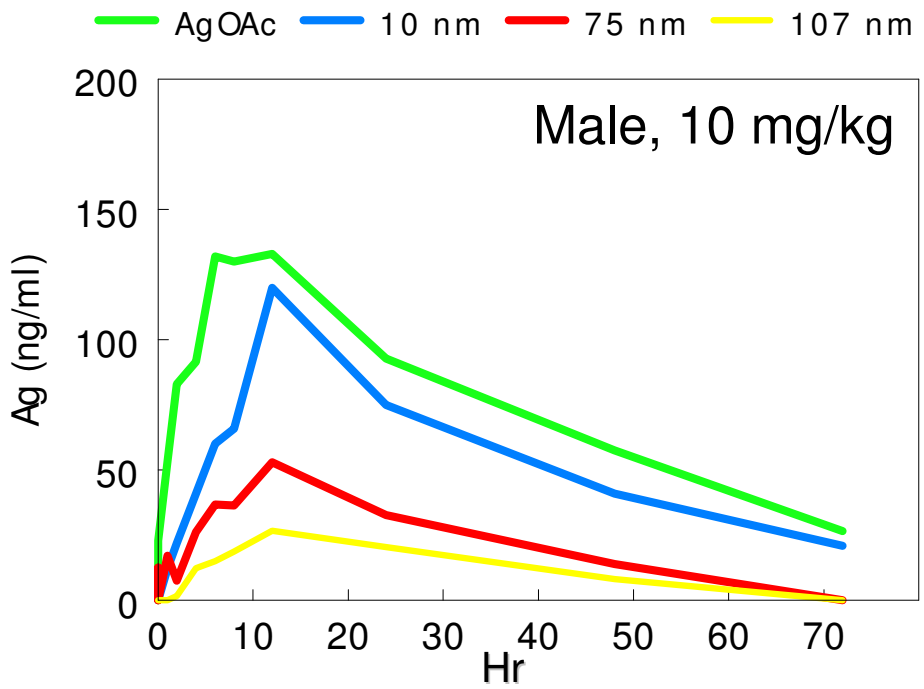




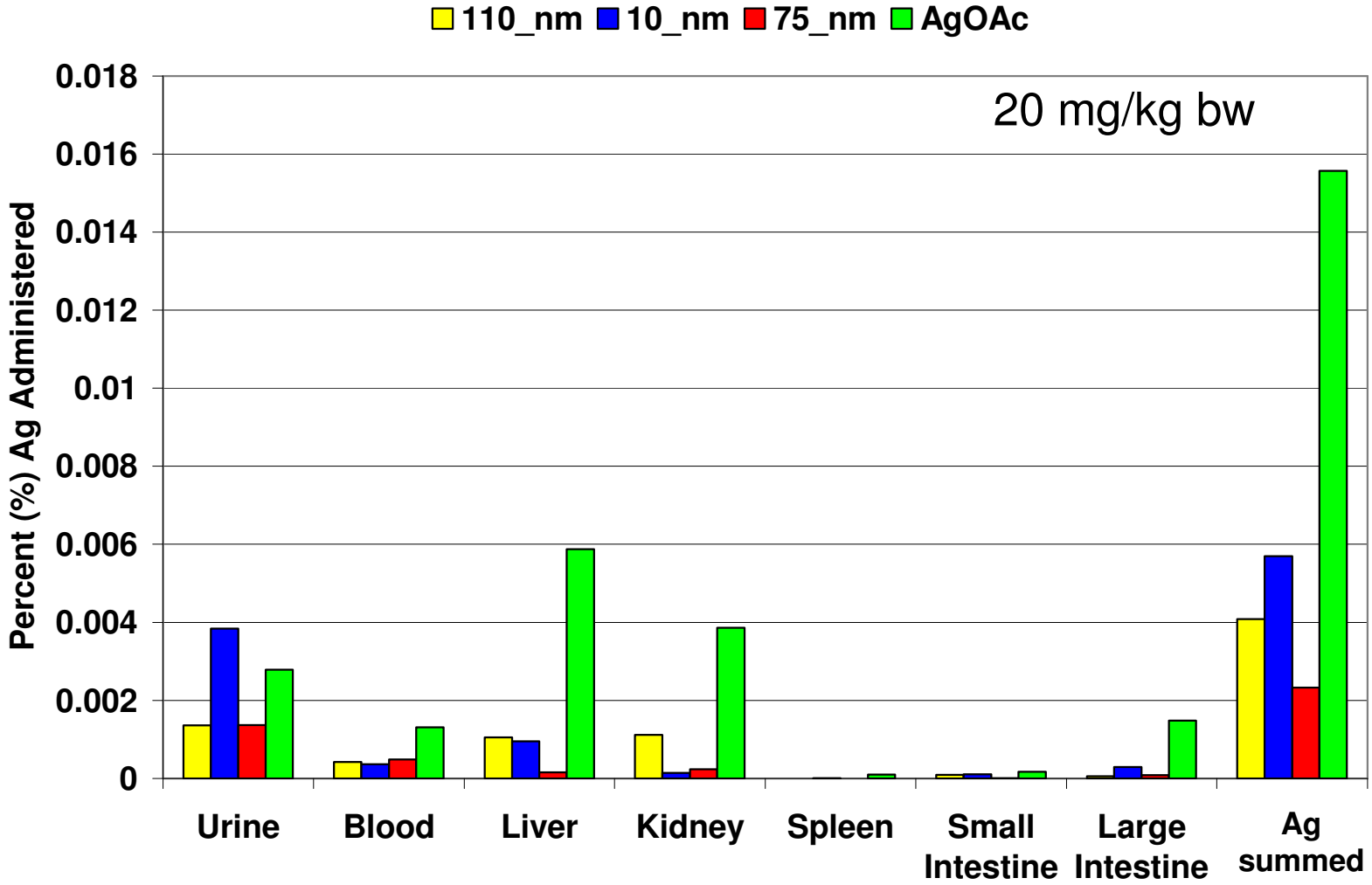
Blood Levels of Silver Following Oral AgNP Administration

- The T_{max} (12 h) and $t_{1/2}$ for distribution (4-5 h) and elimination (24 h) were similar among silver particles
- AUC decreased as particle size increased; males had lower AUCs than females

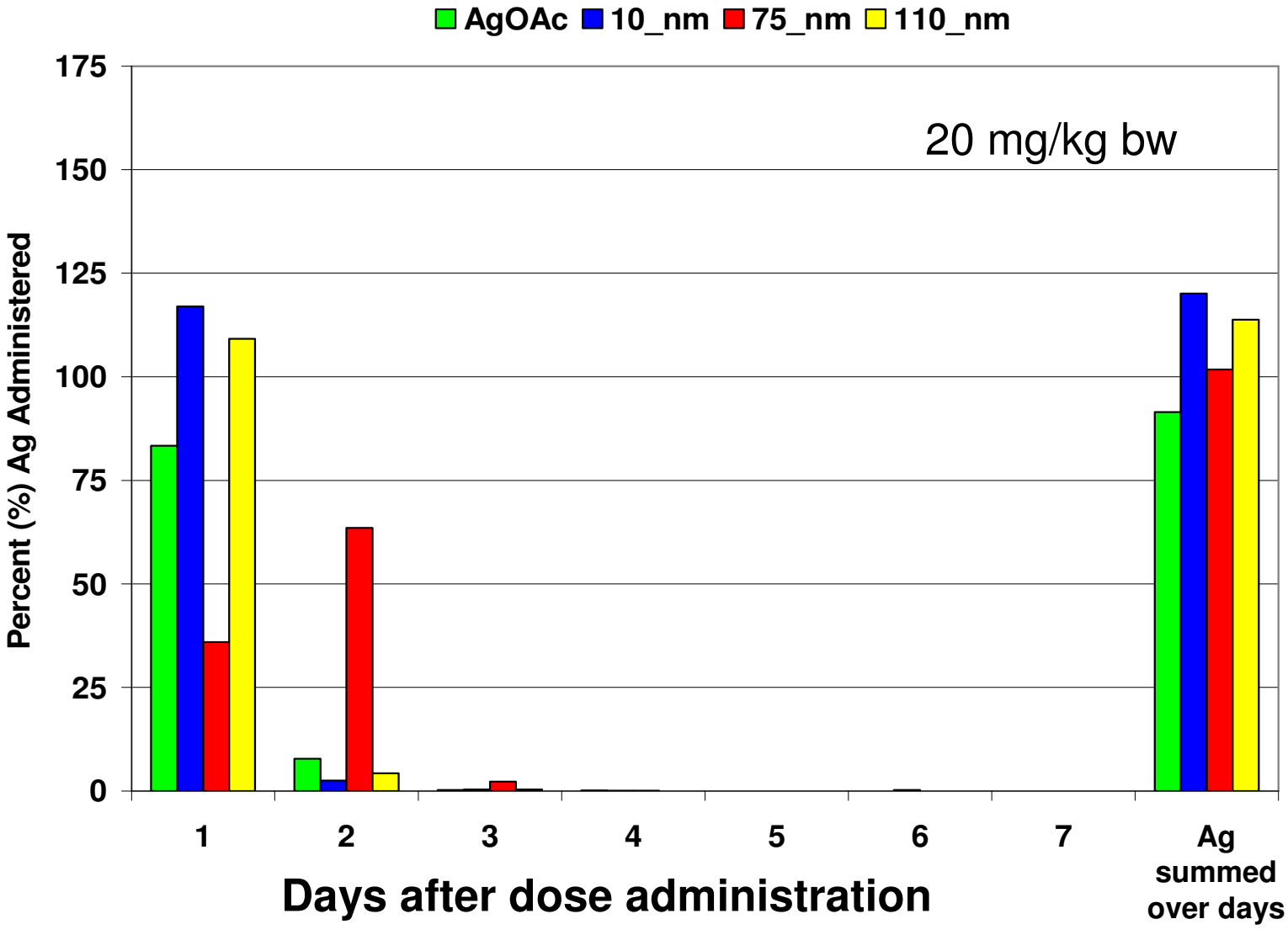




Blood, Urine, and Tissue Deposition of Silver Following Oral Administration



Elimination of Silver Following Oral Administration



Summary of Results

- **Pharmacokinetic Bioavailability**
  - **Maximum concentration for oral administration was constant at 12 h, and half elimination time was 24 h**
  - **AUCs decreased as particle size increased**
  - **Lower AUCs were observed for males than females**
- **ADME**
  - **Minimal absorption following single oral administration**
  - **Trace accumulation in organs, blood, and urine**
  - **Major elimination route via feces**

AgNP 13-Week Study Design

- Sprague Dawley rats (10 animals/sex/treatment)
  - AgNP (10, 75, and 110) or AgOAc
- Administration by oral gavage:
  - 9, 18, and 36 mg AgNP/kg bw; 100, 200, and 400 mg AgOAc/kg body wt
  - Doses divided 2/day, 7 days/week for 13 weeks
- In-life data
  - Weekly water and feed intakes; body weights daily
  - Blood at weeks 1, 4, 8, and 12 via tail vein
    - ICP-MS and micronuclei assays
  - Vaginal cytology

AgNP 13-Week Study Design – Cont'd

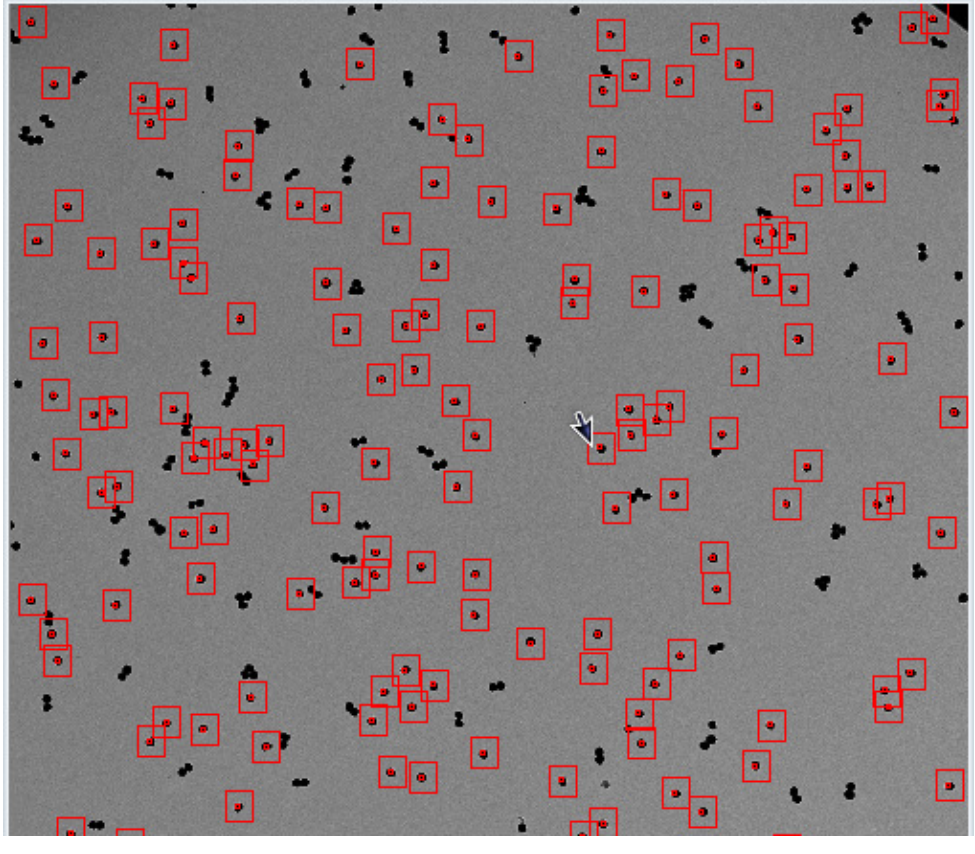
- Terminal Sacrifice
  - Complete necropsy (all animals)
  - Cardiac blood for clinical chemistry and hematology
  - Sperm motility counts
  - Complete histopathology of treatment-related lesions, all controls, highest dose group with 60% survival, and all higher dose groups
  - TEM and ICP-MS (limited tissues)
    - Gastro intestinal tract
      - Site of absorption and transport
      - Effects of exposure on microbiota populations

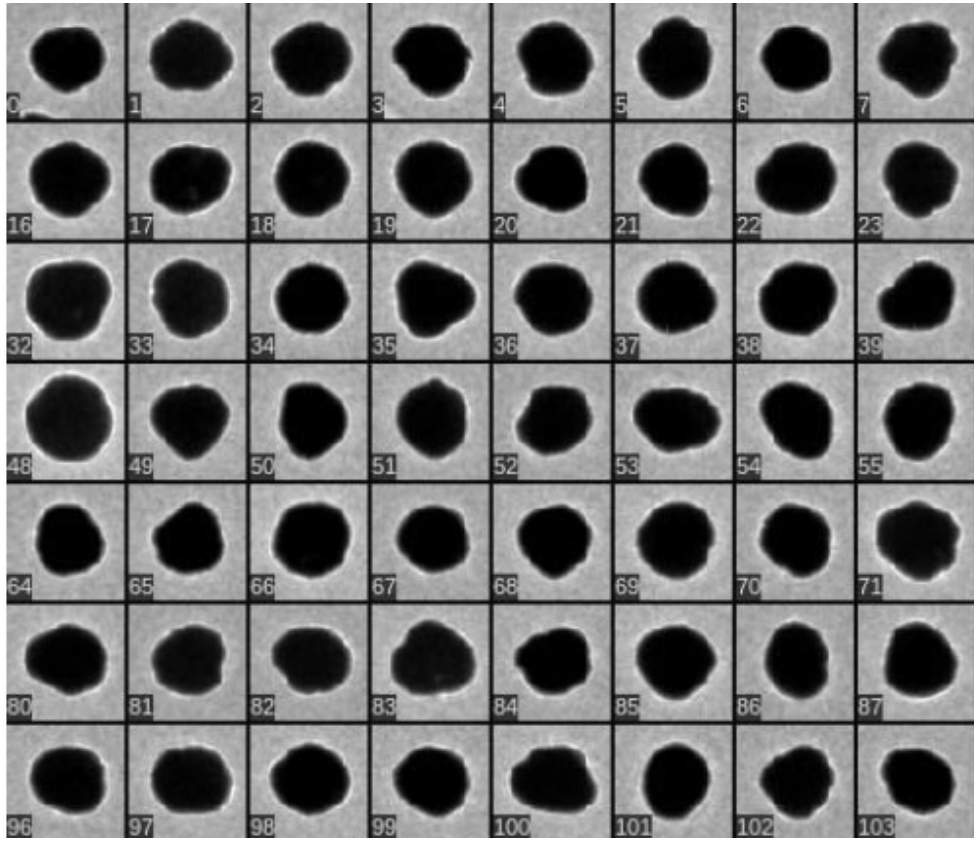
Test Article Characterization

- Test articles (shipments arrive weekly for 22 weeks)
  - TEM for particle size and aspect ratio
  - Dynamic light scattering for particle size, size distribution, and zeta-potential
  - ICP-MS for Ag^{0} and Ag^{+1} concentration by mass
- Dose solutions (doses prepared weekly for 22 weeks)
  - TEM for particle size and aspect ratio
  - Dynamic light scattering for particle size and size distribution
  - ICP-MS for Ag^{0} concentration by mass

Assessment of AgNP by Transmission Electron Microscopy

- Jeol 2100 (200 kV) TEM with Gatan 16 M-pixel CCD
- Particle diameter is measured and the aspect ratio is calculated based on particle width and height.

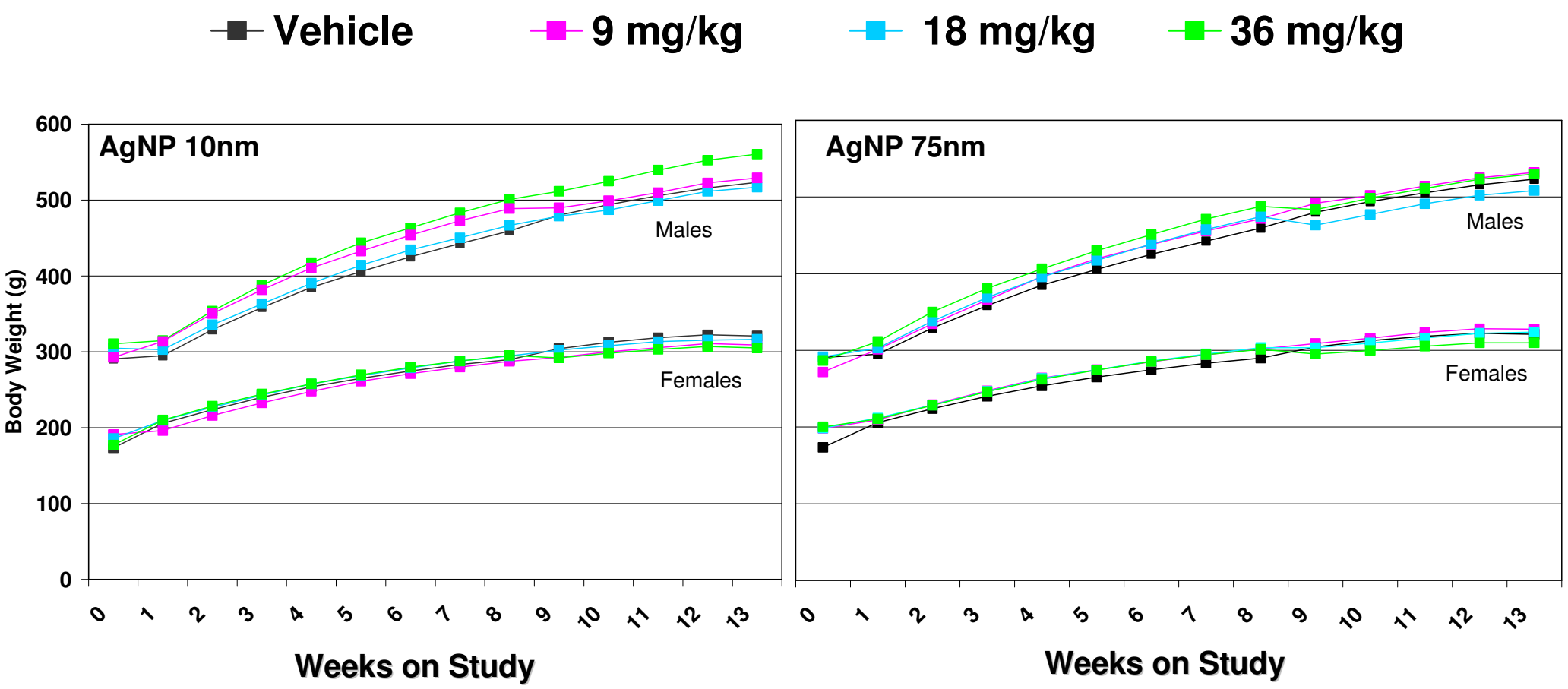




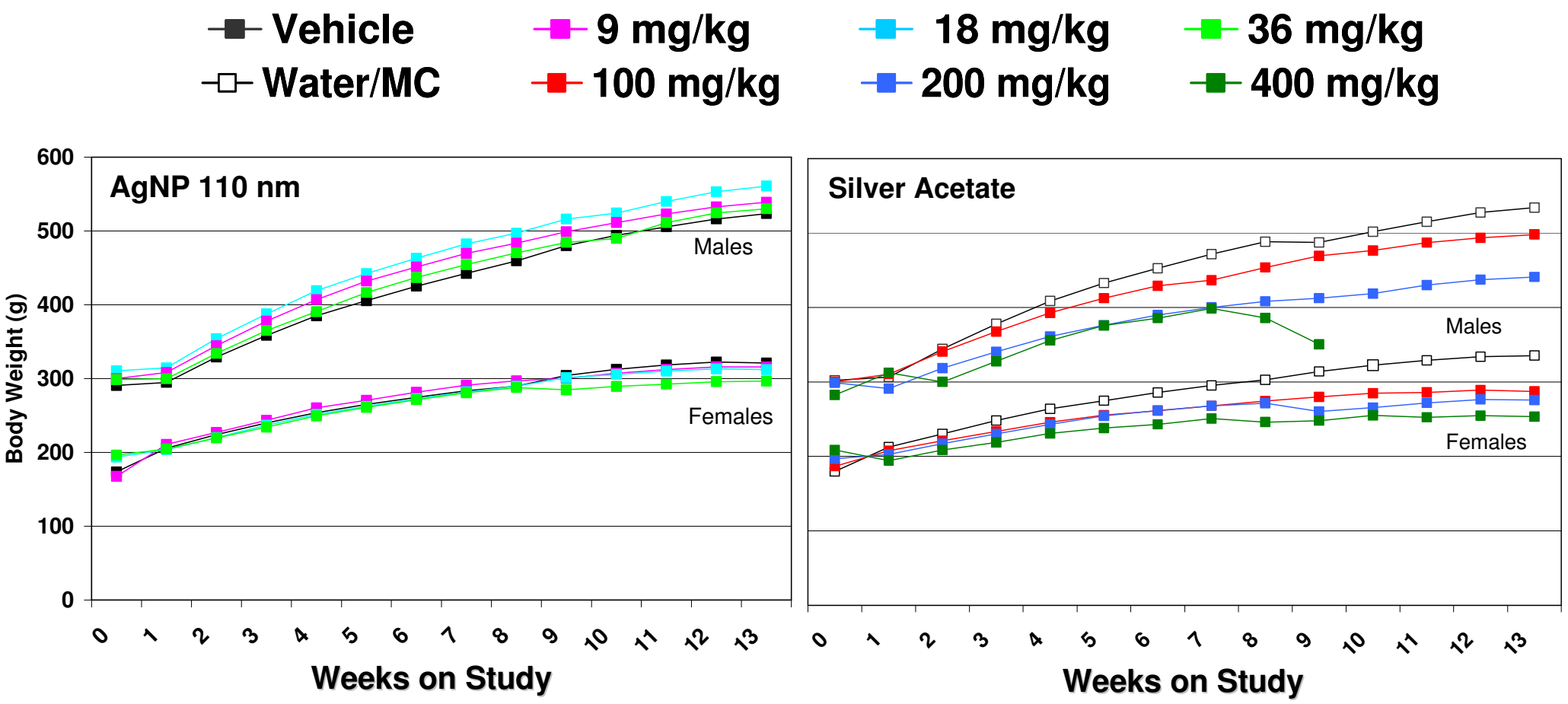
Test Material Characterization by ICP-MS, DLS, and TEM

Sample	Nominal conc. (mg/ml)	Actual mass conc. (mg/ml)	Mean particle size (nm)	Zeta potential	Mean particle diameter	Mean particle aspect ratio
Gold Std			63.5	56.4		
10 nm AgNP	1.0	0.96	17.1	-34.7	8.15	1.09
75 nm AgNP	1.0	1.08	73.6	-46.3	72.1	1.13
110 nm AgNP	1.0	0.99	103.3	-44.1	101.1	1.13
AgOAc	6.46	6.78	-	-		

13-Week Study Body Weights For 10 nm AgNP and 75 nm AgNP



13-Week Study Body Weights For 110 nm AgNP and Silver Acetate



AgNP 13-Week Study Animal Survival

Treatment	Dose (mg/kg body weight/day)	Moribund Removal	
		M	F
Vehicle/0.1% CMC	0	1	
Water/0.1% MC	0		
AgNP 10 nm	9		
	18		1
	36		
AgNP 75 nm	9		
	18		
	36		
AgNP 110 nm	9		1
	18		
	36	1	
AgOAc	100		1
	200		
	400	10	8

Preliminary Findings

- Test articles and dose certifications were within ± 10% target values
- Stability of test articles was confirmed for 60 days
- The oral administration of AgOAc at doses of 200 mg/kg bw and higher induced significant decreases in body weight and increased morbidity in rats
  - Severe gastroenteritis listed as cause of death
- No overt dose response pathologies were observed for AgNPs.
  - Renal disease listed as cause of death for 2 rats exposed to AgNP 110 nm and 1 rat exposed to AgNP 10 nm

Acknowledgements

- Research for this project is funded through interagency agreements (FDA/NCTR IAG # 224-07-0007, NIEHS/NTP IAG # Y1ES1027) between the FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS)/NTP
- The opinions expressed in this presentation are based on preliminary data and may not reflect those of the U.S. FDA