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Supplementary Information

Critical assessment of toxicological effects of ingested nanoparticles

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Table 1S: Summary of literature investigating SiO_2 nanoparticle toxicity *in vitro* since 2012.

SiO_2 NP characteristics	**Dose and treatment time**	**Cell type**	**Major conclusions**	**Ref.**
PEG 6000, PEG 20000, chitosan, and sodium alginate-coated SiO_2 NP, some NP were associated with insulin prior to coating.	50-500 µg/mL, 48 hours	Caco-2 cells	Surface modification made little difference in SiO_2 NP toxicity and all particles displayed very low toxicity in Caco-2 cells up to 500 µg/mL, showing biocompatibility for oral drug delivery.	1
24.3 and 45.3 nm Fe_3O_4-SiO_2 core-shell NP and 27.3 and 52.8 nm $CoFe_2O_4$-SiO_2 core-shell NP, modified with polyelectrolytes and attached DNA fragments	10-20 µg/mL, 24 hours	Caco-2 cells	Tuning of the magnetic SiO_2 NP to change the zeta potential altered internalization of NP by cells and NP cytotoxicity, with observed toxicity at 10 µg/mL. The cell response seems to be dependent on the final surface charge ratio.	2
20, 30, and 100 nm SiO_2 NP, uncoated or modified with COOH	0.6-600 µg/mL, 4-24 hours	Caco-2 and HT-29 cells	The presence of the protein corona ameliorated SiO_2 NP cytotoxicity (apparent at 60 µg/mL), and the corona formed was dependent on the NP properties.	3
12, 40, and 200 nm SiO_2 NP	0.1-500 µg/mL (0.03-156.3 µg/cm^2), 1-72 hours	HT-29 cells	SiO_2 NP can interfere with cellular pathways, influence cell growth, and cause cytotoxicity, although some of these effects were observed beginning at 31.3 µg/cm^2 (100 µg/mL), which are doses higher than may be realistic. Further studies are needed to investigate the fate of NP in the gastrointestinal (GI) tract.	4
14 nm SiO_2 NP, uncoated	0.3125-80 µg/cm^2, 1-24 hours	Caco-2 cells	Undifferentiated cells were more sensitive to SiO_2 NP toxicity and inflammation, with the TC_{50} value determined to be 9 µg/cm^2 after 24-hour exposure, but > 80 µg/cm^2 in differentiated cells, although the undifferentiated cells may be less relevant to *in vivo* systems. Simulated gastrointestinal digestion had a negligible effect on NP toxicity.	5
54.3 nm SiO_2 NP, coated with poly(2,3-hydroxy-	0.1-1 mg/mL, 4	Caco-2 cells	The polymer grafting density on SiO_2 NP determined their cytotoxicity. Highly packed, neutral polymers protected the	6

propylacrylamide), poly(acrylic acid), and poly(2-aminoethylacrylamide)	hours		cells from SiO_2 toxicity but NP with a lower packing density were more cytotoxic (beginning at 0.1 mg/mL and more evident at 0.5 mg/mL). Therefore, the polymer structure, grafting density, and NP core will alter toxicity and biocompatibility.	
12 nm SiO_2 NP	10 μg/cm^2, 24 hours	Caco-2 cells	No cytotoxicity was observed after 24-hour 10 μg/cm^2 SiO_2 NP exposure and susceptibility was not changed after repeated exposure of the cell population to SiO_2 NP. Simulated digestion of the SiO_2 NP prior to cell exposure also did not change NP toxicity. However, SiO_2 NP were internalized by cells and thus it is essential to monitor *in vivo* NP distribution.	7
12 nm SiO_2 NP	10 μg/cm^2, 1-10 days	Caco-2 cells	Continual SiO_2 NP treatment of proliferating cells moderately inhibited cell proliferation at 10 μg/cm^2, but the relevance of this for doses at which SiO_2 would be ingested is unclear.	8
34 and 62 nm RITC-labeled and 15, 32, and 61 nm propidium iodide-labeled fluorescent SiO_2 NP, and 28 nm and 71 nm RITC-SiO_2 NP were modified with polyethylene glycol (PEG) chains	20-500 μg/mL, 4-24 hours	Caco-2 cells	The SensorDish® Reader assay was determined to be a sensitive, rapid, and convenient system to detect toxicity by monitoring the oxygen concentration in solution. All SiO_2 NP except for the largest particles displayed a size-, concentration-, and time-dependent decrease in oxygen consumption, indicating a loss in cellular activity. The smaller the particles and the higher their concentration, the faster the toxicity was apparent, with clear toxicity apparent at 100 μg/mL beginning at 4 hours.	9
Atto 647N dye-incorporated 32 and 83 nm SiO_2 NP	1-200 μg/mL, 5-72 hours	Caco-2 cells	Both sizes of SiO_2 NP were taken up as single particles but agglomerated inside the cell near the nucleus. The smaller NP were internalized and migrated faster than the 83 nm NP and also were localized within the nucleus while the 83 nm NP were not. No cytotoxicity or genotoxicity of SiO_2 NP was observed at 200 μg/mL.	10
20 and 90 nm mesoporous fluorescent SiO_2 NP	10-150 μg/mL, 24	HT-29 cells	The 20 nm SiO_2 NP induced limited cytotoxic and genotoxic effects after 24-hour exposure to cells. The 90 nm SiO_2 NP	11

	hours		induced greater toxicity, with the greatest toxicity observed at 10 μg/mL while higher doses (150 μg/mL) were observed to induce lower cytotoxicity and genotoxicity, showing a complexity to NP toxicity.	
15 and 55 nm SiO_2 NP	4-256 μg/mL, 24 hours	Caco-2 cells	Exposure to 15 nm SiO_2 NP caused cytotoxicity including induction of apoptosis, chromosomal damage, and release of IL-8 at 32 μg/mL, but 55 nm SiO_2 NP did not cause this toxicity. The production of ROS may be involved in the toxicity, and the genotoxic effects must be indirect since the SiO_2 NP did not localize to the nucleus.	12
10-50 nm food additive SiO_2 NP	10-600 μg/mL, 6-72 hours	GES-1 and Caco-2 cells	SiO_2 NP were internalized by cells and inhibited cell growth by cell cycle arrest after 48-hour exposure to doses of 200 μg/mL and higher, but did not change cell morphology or induce apoptosis or necrosis. This suggests that the physiologically relevant doses of SiO_2 NP are likely safe, but long-term and low-dose exposure should be studied further.	13
28 nm Rhodamine 6G/silica and 29 nm QD/silica core/shell SiO_2 NP	100 μg/cm^2, 24 hours	Caco-2 cells	Fluorescent core/shell SiO_2 NP were internalized by cells and localized in aggregates within the cytoplasm but were not found in the nucleus at 100 μg/cm^2 doses.	14
45 nm spherical SiO_2 NP	2.5-15 mg/mL, 24 hours	Caco-2 cells	SiO_2 NP induced a 10-15% decrease in cell viability between 2.5-15 mg/mL concentrations of SiO_2 NP, revealing low cytotoxicity. This indicates that SiO_2 NP could be used as a model drug delivery carrier.	15
15 nm SiO_2 NP	1-243 μg/mL, 4-48 hours	Caco-2 cells	No toxicity, ROS production, or IL-8 generation was observed in cells exposed to SiO_2 NP or SiO_2-doped paint particles at doses up to 243 μg/mL. Although this suggests that paint containing SiO_2 NP will not pose a risk, long-term studies are necessary to further investigate the environmental risk.	16
23.7 nm SiO_2 NP	62.5-1000 μM, 12-24 hours	SW480, DLD-1, and NCM460 cells	Treatment of cells with SiO_2 NP induced ROS production at 250 μM, slightly decreased cell viability at 62.5 μM, and induced minimal production of inflammatory cytokines. From this, SiO_2 NP toxicity was determined to be minimal.	17

			Genetic differences between cell lines affected sensitivity to NP, and thus the cell model is important in determining nanotoxicity.	
47.5, 99, and 176 nm fluorescently-labeled SiO_2 NP	0.1-10 mg/mL, 0.25-6 hours	Caco-2 cells	SiO_2 NP were found to agglomerate in simulated gastrointestinal fluids. No significant penetration of 1 mg/mL SiO_2 NP through the Caco-2 cell monolayer was observed. Cytotoxicity was observed of the 47 nm (0.1 mg/mL) but not 99 or 176 nm SiO_2 NP (up to 10 mg/mL), and NP agglomeration decreased toxicity. NP behavior in the GI tract will be important in determining oral toxicity.	18
25-30 nm unmodified and BSA-coated SiO_2 NP	0-300 µg/mL, 24 hours	Colon-26 (murine colon carcinoma epithelial) and Caco-2 cells	Species played a role in NP cytotoxicity observed, with greater toxicity in Colon-26 cells than Caco-2 cells (EC_{20} toxicity values based on the CCK-8 assay were 59.1 µg/mL in Colon-26 cells and 75.6 µg/mL in Caco-2 cells). BSA-stabilized SiO_2 NP were less toxic than unmodified SiO_2 but both NP were internalized similarly. Gene array analysis revealed involvement of lipid homeostasis, transcription, cell junction, and extracellular matrix pathways, but not oxidative stress.	19
16, 24, and 44 nm SiO_2 NP	1.8-1800 µg/mL, 1-24 hours	Rainbow trout intestinal epithelial cells (RTgut-GC)	Cytotoxicity of SiO_2 NP was dependent on NP sizes, exposure temperature, exposure time, and dosage. The calculated EC_{50} values were greater than 100 µg/mL for all NP sizes, thus revealing a low cytotoxicity of SiO_2 NP for fish *in vitro*.	20

Table 2S: Summary of *in vivo* chronic oral SiO_2 nanoparticle exposure studies since 2012.

SiO_2 NP characteristics	**Dose and duration of exposure**	**Animal model**	**Major conclusions**	**Ref.**

Rat models				
Spherical 12 nm SiO_2 NP	Oral gavage at 489.8, 979.5, and 1959 mg/kg/day SiO_2 NP for 14 days, then oral gavage at 244.9, 489.8, and 979.5 mg/kg/day for 13 weeks.	Five-week-old female Sprague-Dawley rats	There was no significant increase in the Si concentrations in the SiO_2 NP-treated rats and there were no dose-dependent changes to suggest systemic toxicity in the 14-day or 13-week studies.	21
20 and 100 nm SiO_2 NP	Oral administration of 500, 1000, and 2000 mg/kg/day SiO_2 NP for 14 and 90 days.	Six-week-old Sprague-Dawley rats	Neither size of SiO_2 NP had a toxic effect on the organs or tissues studied, so that the no-observed-adverse-effect-level (NOAEL) is greater than 2000 mg/kg, and no target organs were identified.	22
18 nm (NM-200) and 25 nm (NM-203) SiO_2 NP	20 mg/kg/day for 5 days	Sprague-Dawley rats	There was negligible or undetectable deposition of SiO_2 in tissues following 5-day NP administration. Changes were observed in liver, lung, and uterus weights of rats administered both NP sizes at 20 mg/kg, and histopathological changes were observed in spleen of treated rats and in liver for female NM-203-treated rats.	23
20 and 100 nm negatively and positively (modified with L-arginine) charged SiO_2 NP	Oral administration of 1000 mg/kg/day positively charged 20 nm SiO_2 NP and 2000 mg/kg/day negatively charged 20 nm SiO_2 NP for 28 days and 90-day administration of positively charged 20 and 100 nm SiO_2 NP at 1000 mg/kg/day.	Six-week-old rats	The data from this study suggest that the 90-day oral SiO_2 NP administration did not compromise or damage the blood brain barrier and no accumulation of SiO_2 NP in brain tissue was observed by TEM, suggesting no significant neurotoxic effects of SiO_2 NP oral ingestion up to 2000 mg/kg.	24
18.3 and 18.0 nm precipitated and 17.7 and 24.7 nm pyrogenic SiO_2 NP (NM-200, NM-201, NM-202, and	Oral administration of 5, 10, and 20 mg/kg/day SiO_2 NP for three days (at 0, 24, and 45 hours).	Six-eight-week-old male Sprague-Dawley rats	Short-term oral exposure to up to 20 mg/kg SiO_2 NP did not induce DNA damage in various organs of rats. The lack of toxicity may be due to the low bioavailability of SiO_2 NP after short-term exposure and this study cannot rule out genotoxic effects after long-term exposure. Pyrogenic SiO_2 NP did induce a weak increase in	25

NM-203)			micronucleated cells in the colon of rats exposed to the lowest dose (5 mg/kg), which might be due to a higher uptake of NP because of a low agglomeration/aggregation state at the low dose.	
7 nm and 10-25 nm (NM-202) SiO_2 NP	Oral administration of 100, 1000, and 2500 mg/kg/day of 7 nm SiO_2 NP and 100, 500, and 1000 mg/kg/day of NM-202 for 28 days via mixing NP with powdered standard feed pellets and chocolate milk. An 84-day study was performed with 2500 mg/kg/day 7 nm SiO_2 NP and 1000 mg/kg/day NM-202.	Nine-week-old Sprague-Dawley rats	Oral exposure of rats to NM-202 (84-day study, 1000 mg/kg) resulted in mild fibrosis to the liver whereas the 7 nm SiO_2 NP did not, but it is unclear what properties caused this difference. Total SiO_2 content in tissues was lower in the higher-dosed animals (1000 or 2500 mg/kg) than the lower-dosed animals (100 mg/kg), although this difference was not statistically significant. *In vitro* digestion studies revealed stronger gel-like properties of SiO_2 NP in the middle and high dose groups. This implies low gelation of SiO_2 in human intestine at realistic consumer exposure levels, and suggests that future studies should investigate lower doses.	26
15 nm unmodified, PEG-coated, phosphonate-functionalized, and amine-functionalized SiO_2 NP	Oral administration of 1000 mg/kg/day SiO_2 NP for 28 days.	42-day-old Wistar rats	None of the SiO_2 NP preparations induced adverse effects after 28-day administration at 1000 mg/kg. Thus, this suggests no systemic toxicity of SiO_2 NP, but further studies need to develop standardized NP preparation procedures for oral administration.	27
10-25 nm SiO_2 NP (NM-200)	Oral administration of 100, 300, or 1000 mg/kg/day SiO_2 NP to pregnant dams from gestational day 6 (implantation) through gestational day 19 (end of gestation).	12-15-week-old Wistar rats	No maternal or embryo-fetal toxicity was observed after administration of 1000 mg/kg SiO_2 NP to pregnant rats from implantation to the day before expected parturition. Therefore, the NOAEL for SiO_2 NP was determined to be 1000 mg/kg/day.	28
10-15 nm SiO_2 NP	Oral administration of 333.33 mg/kg/day SiO_2 NP for 5 days.	Six-eight-week-old male Wistar rats	Vomiting, loss of appetite, and severe lethargy were observed after 333.33 mg/kg SiO_2 NP treatment to rats. Lung, liver, testis, and kidney were found to be target	29

			organs of SiO_2 NP. Based on the toxicity observed in this short-term study, safety guidelines are needed for those who work directly with SiO_2 NP.	
20 and 100 nm colloidal SiO_2 NP	Oral administration of 500, 1000, or 2000 mg/kg/day 20 nm SiO_2 NP for 28 days.	Six-week-old Sprague-Dawley rats	Only a small proportion (6.25-12.53%) of the administered SiO_2 NP were absorbed into the blood after 28-day administration, and the absorbed NP were eliminated from plasma within 21 hours. Thus, it was concluded that SiO_2 NP have low chronic oral toxicity potential.	30
Mouse models				
70, 300, or 1000 nm SiO_2 particles; 70 nm NP were additionally modified with carboxyl or amine groups	Oral administration of 2.5 mg/mouse/day SiO_2 NP for 28 days for each particle type.	Six-week-old female BALB/c mice	The SiO_2 surface properties determined their intestinal absorption based on everted gut sac studies in Wistar rats, but none of the particles tested induced toxicity after 28-day oral administration of 2.5 mg/mouse/day.	31
32 nm Rhodamine 800/SiO_2, 28 nm Rhodamine 6G/SiO_2, and 29 nm QD/SiO_2 core/shell NP	Oral administration of Rhodamine 800/SiO_2 and Rhodamine 6G/SiO_2 NP at 1 mg/mouse/day (~34.7 mg/kg, experiment 1) and Rhodamine 6G/SiO_2 NP at 0.1 mg/mouse/day (~6.4 mg/kg) and QD/SiO_2 NP at 0.67 mg/mouse/day (~43.0 mg/kg, experiment 2) for 4 days.	Eight-twelve-week-old female SKH1-E mice	Rhodamine 6G/SiO_2 and QD/SiO_2 NP were detected by fluorescence confocal microscopy and ICP-MS after 4-day oral administration at 1 mg/mouse Rhodamine 6G/silica and 0.67 mg/mouse QD/silica in gastrointestinal tract organs as well as the non-gastrointestinal tract tissues of kidney, lung, brain, spleen, and liver, suggesting that SiO_2 NP can enter systemic circulation and may accumulate in tissues. No overt toxicity was observed during this NP administration.	14
Polychlorinated biphenyl (PCB) 153-labeled SiO_2 NP (hydrodynamic diameter of 246.7	Oral administration of 5 ng PCB153/g body weight bound to 1.04×10^5 SiO_2 NP daily for 30 days.	12-week-old C57BL/6 male mice	Treatment with PCB153-SiO_2 NP resulted in a significantly higher accumulation of PCB153 in adipose tissue than administration of PCB153 only. This 30-day administration also enhanced leukocyte aggregation in brain vessels and perivascular space. Thus, modification	32

nm in water)			of "inert" NP such as SiO_2 with other environmental chemicals may significantly influence toxicity.	
Other models				
50 nm SiO_2 NP, unlabeled, rhodamine B-labeled, and FITC-labeled.	The OP50 (bacterial lawn *E. coli*) was supplemented with 2.5 mg/L SiO_2 NP suspended in water for up to 7 days.	*Caenorhabditis elegans* wild-type N2	Premature aging phenotypes were observed in *C. elegans* treated with 2.5 mg/L SiO_2 NP. Protein homeostasis was shifted to more insoluble ubiquitinated proteins and formation of SDS-resistant protein aggregates. SiO_2 NP accumulation in the pharynx and vulva was correlated with premature reduction of pharyngeal pumping and egg laying behavior. The *C. elegans* model has the potential to be used to screen nanomaterials and investigate the NP biointerface.	33
22 nm colloidal SiO_2 NP	Oral exposure to SiO_2 NP at 34 and 340 µg/L and 34, 170, and 340 mg/L in sugar water for drinking.	Bumblebee workers (*Bombus terrestris*)	No lethal effects were observed even at the highest dose of SiO_2 NP. Concentrations of 34 mg/L or greater caused sublethal effects which resulted in a loss of bumblebee reproduction, but concentrations < 340 µg/L were harmless. Histology revealed serious damage to the midgut epithelium, suggesting that the NP interfered with food uptake. Future studies should investigate translocation of NP to other tissues.	34
20-30 nm SiO_2 NP	Exposure of larvae to solutions of 1, 10, and 100 µg/mL SiO_2 NP in 5% sucrose for 12-36 hours.	Wild type *Drosophila melanogaster*	This study revealed membrane destabilization and increased cellular stress and cell death in the midgut tissue of *Drosophila* larvae from ingestion of ≥ 10 µg/mL SiO_2 NP. This suggests that *Drosophila* can be used as a model organism to study adverse effects of NP.	35

Figure 3S: Summary of studies investigating TiO_2 nanoparticle toxicity *in vitro* since 2012.

TiO_2 NP characteristics	**Dose and treatment**	**Cell type**	**Major conclusions**	**Ref.**

	time			
29.8 nm Aeroxide P25 TiO_2 NP	1 pg/L – 1 g/L, 24 hours	NCM460 (human colon mucosal epithelial cells)	Significant cell death was observed at 1 g/L TiO_2 NP, but no cytotoxicity was observed at drinking water-relevant concentrations less than 100 µg/L. However, it is important to prevent persistence and accumulation of NP in water supplies.	36
21 nm Aeroxide P25 TiO_2 NP	0.1-100 mg/L, 4-48 hours	Caco-2 and SW480 cells	Significant cell death was induced in SW480 cells treated with 100 mg/L TiO_2 NP in buffered synthetic freshwater but not in cell culture media. The absence of toxicity in Caco-2 cells indicates that not all intestinal epithelial cells may respond to TiO_2 NP the same way. No ROS generation was detected, but NP treatment did induce IL-8 generation.	37
40-300 nm TiO_2 NP isolated from chewing gum and Aeroxide P25 TiO_2 NP	10-200 µg/mL, 24 hours	GES-1 and Caco-2 cells	It was found that > 93% of TiO_2 particles in six brands of chewing gum were < 200 nm, and 18-44% were < 100 nm. After simulated digestion, the nano-sized fraction of TiO_2 decreased significantly. Isolated TiO_2 NP induced little toxicity in GES-1 and Caco-2 cells at up to 200 µg/mL, but *in vivo* low-dose studies are still needed.	38
< 25 nm TiO_2 NP	1-20 µg/cm^2 (5-100 µg/mL), 6-24 hours	Caco-2 cells	Six-hour 2.5 µg/cm^2 TiO_2 NP treatment increased intracellular ROS levels, but did not increase IL-8 release. After 24-hour TiO_2 exposure, ROS levels were lower than after 6 hours, suggesting that Caco-2 cells were able to maintain their antioxidant potential after TiO_2 treatment.	39
122 nm E171 (food-grade TiO_2) and 141 nm TiO_2 isolated from chewing gum	0.1-10 µg/cm^2 (0.35-35 µg/mL), 24 hours	C2BBe1 cells	Food-grade TiO_2 (0.1 µg/cm^2) disrupted the brush border and induced the loss of approximately 42% of microvilli, indicating a need for further studies to better understand this response.	40
10×50 nm TiO_2 core coated with $Al(OH)_3$ and polydimethylsiloxane	10-100 µg/mL, 4-72 hours	Caco-2 cells	No toxicity was observed after up to 72-hour exposure to 100 µg/mL TiO_2 NP. No TiO_2 penetration into cells was observed by TEM, and no changes were observed in gene expression after 10 µg/mL TiO_2 NP exposure. ROS	41

(PDMS, T-Lite™ SF from BASF)			generation was detected only on unmodified TiO_2 NP, and the PDMS coating is likely protective. This suggests that the TiO_2 NP are likely not toxic and may not penetrate the body.	
25.2 nm 77% anatase/23% rutile (Aeroxide P25) TiO_2 NP, 21.9 nm 90% anatase/10% rutile TiO_2 NP, 6.7 nm anatase TiO_2 NP, and 3.94 nm anatase TiO_2 NP, compared to fine 215 nm anatase TiO_2	10-160 $\mu g/cm^2$, 4-24 hours	Caco-2 cells	Cytotoxicity of TiO_2 NP increased with increasing specific surface area and anatase/rutile samples were more toxic than anatase only. The anatase/rutile TiO_2 NP increased LDH release at 80 $\mu g/cm^2$ while the anatase only NP did not. However, all the NP induced a decrease in metabolic activity after 24-hour exposure to 80 $\mu g/cm^2$ NP. There was no indication that the toxicity of TiO_2 NP was due to oxidative stress.	42
22.8 nm 25% rutile/75% anatase TiO_2 NP (Aeroxide P25), 16.4 nm anatase TiO_2 NP, and 30.8 nm rutile TiO_2 NP compared to 103.2 nm bulk TiO_2	1-10 mg/L, 24 hours	Caco-2 cells	Caco-2 cells internalized and accumulated Ti after TiO_2 NP exposure, seemingly through active NP uptake. The anatase TiO_2 NP were internalized faster than rutile TiO_2, suggesting that different forms of TiO_2 will need to be regulated differently. Little LDH leakage was observed at 1 mg/L TiO_2 NP, but electrolyte composition (K^+, Na^+, Ca^{2+}, Mg^{2+}) was disturbed at this dose and further study should be made of potential NP interference with essential minerals.	43
21 nm TiO_2 NP (Aeroxide P25)	10 $\mu g/cm^2$, 24 hours	C2BBe1 cells	Exposure of C2BBe1 cells to 10 $\mu g/cm^2$ TiO_2 NP for 24 hours did not induce toxicity, and repeated exposure of the cell population to TiO_2 NP (once a week for 24 hours) did not change the cell response. *In vitro* digestion of the NP prior to cell exposure caused little change in toxicity. Further studies should determine the potential for absorption and distribution of TiO_2 NP *in vivo*.	7
21 nm TiO_2 NP (Aeroxide P25)	1-10 $\mu g/cm^2$, 24 hours	C2BBe1 cells	TiO_2 NP induced a moderate inhibition of cell proliferation at a dose of 10 $\mu g/cm^2$. Although this should be further investigated *in vivo*, it is likely that this	8

			effect, not apparent at 1 μg/cm^2, will be irrelevant at human oral exposure levels.	
20-60 nm TiO_2 NP, anatase	1-20 μg/cm^2 (6.4-128 μg/mL), 1-24 hours	Caco-2 cells	TiO_2 NP increased 8-oxodG levels in Caco-2 cells after 6-hour exposure to 1 μg/cm^2 TiO_2 NP. There was little formation of micronuclei or DNA damage induced by TiO_2, but what damage did occur appeared to be well-repaired, as evidenced by the decrease in 8-oxodG over time and increased expression of the DNA glycosylase hOGG1.	44
12 nm 95% anatase/5% rutile TiO_2 NP and 22 nm 10% anatase/90% rutile TiO_2 NP	50-200 μg/mL, 6-48 hours	Caco-2 cells	TiO_2 NP induced up-regulation of multiple nutrient transporters and efflux pumps from the ABC transporter family in Caco-2 cells at 50 μg/mL. This is correlated to the misbalance of cellular redox systems. However, TiO_2 NP did not cause mortality or DNA damage in cells. Low-dose studies need to be performed to further investigate effects on nutrient and drug absorption.	45
19 nm TiO_2 NP	1-243 μg/mL, 4-48 hours	Caco-2 cells	No toxicity, ROS production, or IL-8 generation was observed from TiO_2 NP or TiO_2-doped paint particles at doses up to 243 μg/mL. This suggests that paint-containing TiO_2 will not cause an acute risk, but long-term studies are still needed.	16
27.6 nm TiO_2 NP	62.5-1000 μM, 12-24 hours	SW480, DLD-1, and NCM460 cells	TiO_2 NP toxicity was determined to be minimal based on induction of ROS production at 250 μM, a slight decrease in cell viability at 62.5 μM, and minimal production of inflammatory cytokines. Genetic differences between cell lines affected sensitivity to NP, indicating that the cell model is important in determining nanotoxicity.	17
10 nm anatase, 70 nm rutile, and 1.8 μm rutile TiO_2 particles	50-100 μg/mL, 3 hours	Caco-2 cells	Transport studies of TiO_2 NP through Caco-2 transwells revealed transport of < 0.05% of the TiO_2 dose at all concentrations and with all particle sizes.	46
11.2, 14.4, and 144.3 nm anatase TiO_2 NP	40 μg/cm^2, 3 hours-25 days	Caco-2 cells	Exposure to 40 μg/cm^2 TiO_2 NP was observed to transiently activate inflammation (through NF-κB and	47

			p38 MAPK pathways) but did not affect Caco-2 cell differentiation. The 11.2 and 14.4 nm TiO_2 NP exhibited the greatest effect, and thus caution is warranted in the use of TiO_2 NP < 20 nm.	
99 nm food additive TiO_2 and 26 nm TiO_2 NP, both anatase	50-200 μg/mL, 2-24 hours	Caco-2 cells, undifferentiated and differentiated	TiO_2 NP used were internalized by Caco-2 cells, but there was little overall TiO_2 NP toxicity. NP pretreated with digestive fluids prior to cell exposure induced greater toxicity at 50 and 200 μg/mL, inhibiting cell growth and also cellular NP uptake in undifferentiated cells. Despite ROS generation, oxidative stress and cell uptake did not fully explain the mechanism of TiO_2 NP cytotoxicity. Little TiO_2 was transported through the Caco-2 monolayer, suggesting that the absorption of TiO_2 NP *in vivo* may be low.	48
18 nm spherical TiO_2 NP	100 μg/mL, 24-48 hours	Caco-2 cells, differentiated	There was very little (< 0.4%) TiO_2 NP transport through the Caco-2 monolayer grown on inserts at a dose of 100 μg/mL TiO_2, suggesting a low oral absorption *in vivo*. Future studies should focus on the NP properties that determine oral absorption.	49
< 40 nm TiO_2 NP, anatase and rutile mixture	1-1000 μg/mL, 12 hours-10 days	C2BBe1 cells, differentiated	TiO_2 NP were transported across the epithelial cell monolayer by transcytosis at 10 μg/mL TiO_2. No cell death was induced by up to 1000 μg/mL TiO_2 NP. However, 10 μg/mL TiO_2 disrupted microvilli organization on cells and increased intracellular-free calcium. Thus, attention should be paid to non-lethal effects of TiO_2 NP exposure.	50
10 nm×200 nm×7000 nm TiO_2 nanobelts, 100% anatase	10-100 μg/mL, 1-24 hours	Caco-2/HT29-MTX co-cultures, differentiated	Only low levels of TiO_2 nanobelt-mediated toxicity were observed in Caco-2/HT29-MTX co-cultures after 24-hour exposure to 100 μg/mL TiO_2. However, gene expression analysis after 24-hour exposure to 10 or 100 μg/mL TiO_2 nanobelts revealed induction of a stress response including inflammation and the NF-κB signaling pathway (apoptosis, cell cycle arrest, DNA	51

			replication stress, and genomic instability).	
5 nm TiO_2 NP	50-200 µg/mL, 12-48 hours	Caco-2 monocultures, Caco-2 and HT29-MTX cell co-cultures, and Caco-2/Raji B cell co-cultures (M cell model)	TiO_2 NP accumulation was greater in goblet and M cells than enterocytes, and translocation was only observed in the M cell model at 50 µg/mL. TiO_2 NP accumulation induced a disruption of cell junctions via deregulation of genes involved in epithelial structure maintenance, leading to increased paracellular transport of TiO_2 NP if cell junctions are not repaired.	52
15 nm anatase, 25 nm 80% anatase/20% rutile, 40-50 nm rutile, and up to 5 µm rutile TiO_2 particles	10-250 µg/mL, 24 hours	Caco-2 monocultures and Caco-2/Raji B cell co-cultures (M cell model)	Although some decrease in cell viability was observed at 10 µg/mL TiO_2 (greater at larger particle sizes) in Caco-2 cells, no TiO_2 particles were observed to cross the Caco-2/M cell monolayer and this suggests that there would be little TiO_2 absorption *in vivo*.	53
7-10 nm TiO_2 NP (NM-101)	1.25-625 µg/cm^2, 24 hours	Caco-2 cells cultured with THP-1 cells (macrophages) and MUTZ-3 cells (dendritic cells) embedded in collagen	No toxicity of TiO_2 NP was observed after exposure to the co-culture model or a Caco-2 cell monoculture up to 625 µg/cm^2. However, this co-culture model provides a new approach for studying intestinal inflammation *in vitro*.	54
21 nm Aeroxide P25 TiO_2 NP, 147 nm bulk TiO_2	1 mg/L, 4 hours	Rainbow trout (*Oncorhynchus mykiss*) intestines were isolated and everted for NP exposure.	Exposure to bulk or nano-TiO_2 can result in an increase in the rainbow trout intestinal Ti concentration. Ti uptake was ~ 1-3 nmol/g/h in perfused intestine. Ti uptake was faster when intestine was exposed to NP rather than bulk TiO_2.	55

Table 4S: Summary of *in vivo* chronic oral TiO_2 nanoparticle exposure studies since 2012.

TiO_2 NP characteristics	**Dose and duration of exposure**	**Animal model**	**Major conclusions**	**Ref.**
Rat models				
< 100 nm TiO_2	Oral administration of	Male albino	TiO_2 NP administration to rats resulted in dose-dependent	56

NP	600 or 1000 mg/kg/day TiO_2 NP for 5 days. Some rats were co-administered 200 mg/kg/day quercetin or idebenone along with TiO_2 NP.	rats	renal toxicity as evidenced by elevation of serum renal function biomarkers (urea, creatinine, and uric acid), inflammatory mediators (TNF-α, IL-6, and CRP), IgG, VEGF, and NO. Toxicity was also confirmed by histological analysis. Treatment of rats with quercetin or idebenone was most helpful in alleviating the damage of TiO_2 NP in the low-dose groups (600 mg/kg/day), which may be due to their ability to neutralize the generated ROS and inhibit inflammatory cytokines induced by the NP.	
75 nm anatase TiO_2 NP	Oral administration of 10, 50, and 200 mg/kg/day for 30 days.	Nine-week-old male Sprague-Dawley rats	Oral administration of 50 mg/kg TiO_2 NP to rats induced genotoxicity as evidenced by double strand DNA breaks in bone marrow cells. However, no damage to chromosomes or mitotic apparatus was observed. Further studies are needed to understand the mechanism of TiO_2 NP-induced genotoxicity.	57
21 nm 80% anatase/20% rutile TiO_2 NP	Oral administration of 520.8, 1041.5, and 2083 mg/kg/day for 14 days, and 260.4, 520.8, and 1041.5 mg/kg/day for 12 weeks.	Seven-week-old Sprague-Dawley rats	Low absorption of TiO_2 NP was observed after oral administration to rats for 13 weeks, even at a dose of 1041.5 mg/kg/day with little accumulation of Ti in organs or urine and very high concentrations in feces.	58
21 nm TiO_2 NP	Oral administration of 20 mg/mL TiO_2 NP at 0.1 mg/10 g/day for 7 days from days 6 to 12 of gestation.	Pregnant albino Wistar Han rats and offspring	Oral administration of TiO_2 NP to pregnant rats revealed the presence of TiO_2 in both maternal and neonatal pulmonary tissues. In the lungs of the mothers, there were abnormal lamellar inclusions, macrophage and inflammatory cell infiltrates, and pneumocytic apoptosis. There were inflammatory lesions and saccular maldevelopment in neonatal lungs which may increase the risk of respiratory disorders later in life.	59
6 nm anatase (NM-101), 20 nm anatase (NM-102), 20	Oral administration of 2.3 mg TiO_2 NP/animal/day for 5 days (6.8-8.5	Nine-ten-week-old Wistar rats	The TiO_2 NP orally administered to rats showed very limited bioavailability, although some livers and mesenteric lymph nodes of treated rats displayed increased levels of Ti, suggesting that TiO_2 uptake is possible. However, the	60

nm rutile (NM-103), and 20 nm rutile (NM-104) TiO_2 NP	mg/kg/day for male rats and 10.9-12.0 mg/kg/day for females).		intravenous studies suggested slow elimination of Ti from tissues, so further studies are necessary to investigate accumulation.	
Anatase, rutile, and E171 food-grade TiO_2 NP	Intragastric administration of 1 or 100 mg/kg/day TiO_2 NP for 28 days.	Male Wistar rats	The oral administration of 100 mg/kg of rutile TiO_2 NP for 28 days caused accumulation of Ti in the liver, showing the possibility for absorption of TiO_2 NP upon oral ingestion.	61
75 nm anatase TiO_2 NP	Oral administration of 10, 50, and 200 mg/kg/day TiO_2 NP for 30 days.	Three-week-old (young) and eight-week-old (adult) male Sprague-Dawley rats	Young rats were more susceptible to TiO_2 NP exposure than adults. In young rats, TiO_2 NP induced liver and heart injuries and non-allergic mast cell activation in the stomach. In adult animals, only slight liver and kidney injury and decreased intestinal permeability and blood molybdenum contents were induced. TiO_2 NP were able to induce increased GSH/GSSG ratios (oxidative stress) in young and adult rats via an enhancement in glucose and GSH levels in young rats, but via reduction in glutathione peroxidase activity and GSSG levels in adults. Thus, we should consider age when regulating TiO_2 NP intake, particularly as TiO_2 is often ingested with glucose.	62
24 nm TiO_2 NP	Oral administration of 2, 10, and 50 mg/kg/day TiO_2 NP for 30 or 90 days, and administration of the same TiO_2 doses in addition to 1.8 g/kg glucose.	Four-week-old Sprague-Dawley rats	Oral exposure of young rats to TiO_2 NP or TiO_2 NP + glucose were able to induce liver, kidney, and heart injuries and changes in red and white blood cell counts in a dose, time, and gender-dependent manner. Toxicity was more obvious in the presence of glucose due to toxicity of excess glucose alone and/or the toxicological interactions between TiO_2 NP and glucose. Antagonistic effects of TiO_2 NP with glucose were evident in red and white blood cell parameters, but interactions between TiO_2 NP and glucose in liver and heart were either synergistic or antagonistic in different exposure scenarios. Synergistic toxicity could be due to adsorption of glucose on the TiO_2 surface, which facilitated cellular glucose uptake. Antagonistic effects may be due to promotion of TiO_2 NP aggregation by glucose. The effects of glucose with TiO_2 are	63

			relevant since TiO_2 NP are especially used in sweets.	
< 25 nm anatase TiO_2 NP	Oral administration of 1 or 2 mg/kg/day TiO_2 NP for 5 days.	60-day-old Sprague-Dawley rats	This five-day administration of TiO_2 NP revealed that endocrine-active tissues are targets of 1 or 2 mg/kg TiO_2 NP toxicity, but there was only limited tissue deposition and damage observed in the spleen. Thus, sex-endocrine effects should be considered in the safety assessment of NP.	64
5.5 nm anatase (NM-101), 21.7 nm anatase (NM-102), 24.7 nm rutile (NM-103), 25.0 nm rutile (NM-104), and 24.3 nm 84% anatase/16% rutile (NM-105) TiO_2 NP	Oral administration of 2.3 mg TiO_2 NP/rat (10.2-11.4 mg/kg/day for males and 13.1-15.2 mg/kg/day for females) for 5 days.	Wistar rats	Only some incidental Ti was detected in liver and spleen after oral administration of TiO_2 NP, indicating low absorption, although absorption was shown to be possible. There was also some minimal uptake of NM-104 and NM-105 into the mesenteric lymph nodes.	23
Mouse models				
21 nm anatase TiO_2 NP	Oral administration of 150 mg/kg/day TiO_2 NP for 2 weeks. TiO_2-treated mice were also administered idebenone, carnosine, vitamin E, or all three daily for one month immediately following TiO_2 treatment.	Male albino mice	A two-week administration of 150 mg/kg/day TiO_2 NP to mice induced oxidative stress, inflammation, apoptosis, and DNA damage, which led to severe liver injury. Administration of idebenone, carnosine, and vitamin E alleviated the toxicity of TiO_2, with the combination of all three having the most effect, and these agents may be able to be used to protect against TiO_2 NP toxicity in the future.	65
25 nm anatase TiO_2 NP	Oral administration of 10, 50, or 250 mg/kg/day for 42 days.	Four-week-old (pubertal) male Kunming mice	Oral TiO_2 NP exposure to mice caused an increase in spermatozoa abnormalities in the epididymides and decreased layers of spermatogenic cells and vacuoles in the seminiferous tubules. Serum testosterone levels were also decreased after	66

			TiO_2 NP exposure in addition to a reduction in expression of 17β-hydroxysteroid dehydrogenase and P450 17α-hydroxysteroid dehydrogenase in the testis. The expression of cytochrome P450-19, needed to convert testosterone to estradiol, increased. These effects were observed beginning at a dose of 10 mg/kg/day, but especially at 50 and 250 mg/kg/day. This indicates that TiO_2 NP exposure can influence both synthesis and conversion of testosterone, which may lead to the observed reduction of spermatogenesis.	
66 nm mostly anatase TiO_2 NP and 260 nm anatase TiO_2 NP	Oral administration of 100 mg/kg/day TiO_2 NP for 10 days.	Male BL57/6 mice	Oral administration of TiO_2 to mice induced the increase of $CD4^+$ T cells in the duodenum, jejunum, and ileum. There was also a significant increase in the inflammatory cytokines IL-12, IL-4, IL-23, TNF-α, IFN-γ, and TGF-β, particularly in the ileum. This indicates that TiO_2 NP cause a T_H1-mediated inflammatory response in the small intestine at a 100 mg/kg dose of TiO_2. Little difference was observed between the smaller and larger nano-sized particles.	67
5-6 nm anatase TiO_2 NP	Nasal administration of 2.5, 5, and 10 mg/kg/day TiO_2 NP for 90 days.	Five-six-week-old female CD-1 mice	Peroral administration of TiO_2 NP caused severe pathological changes in the hippocampus, spatial recognition impairment, and significant long-term potentiation reduction and down-regulation of N-methyl-D-aspartate receptor subunit expression. TiO_2 NP administration also inhibited CaMKIV, CREB-1, CREB-2, and FosB/DFosB in mouse hippocampal tissues. Thus, further study should be done on the effects of TiO_2 NP to the central nervous system.	68
25.64 nm anatase TiO_2 NP	Oral administration of 64 and 320 mg/kg/day TiO_2 NP for 14 weeks.	Male CD-1 mice	Oral administration of TiO_2 NP resulted in oral absorption and accumulation in the liver, spleen, kidney, pancreas, and small intestine. This accumulation increased ROS levels in tissue, likely activating the inflammatory response and MAPK pathways, and thus causing the observed increase in plasma glucose in mice and insulin resistance.	69
50-75 nm TiO_2 NP, mixture of	Oral administration of 500 mg/kg/day TiO_2	Six-week-old male *Swiss*	Oral administration of TiO_2 NP induced an increase in ROS levels in erythrocytes, liver, and brain, and altered antioxidant	70

rutile and anatase	NP for 21 days.	*albino* mice	enzyme activities. There was also an increase in dopamine and norepinephrine in the brain cerebral cortex, suggesting a neurotoxic potential of the NP. The inhibition of CuZnSOD and MnSOD (markers of oxidative stress) supported this oxidative stress mechanism, and NP were found by TEM to localize within cells in the liver and brain. Thus, oxidative stress appears to be a main mechanism of TiO_2 NP toxicity.	
< 25 nm anatase TiO_2 NP	Oral administration of 1.25-250 mg/kg/day for 28 days.	Female $B_6C_3F_1$ mice	Oral administration of TiO_2 NP at up to 250 mg/kg/day for 28 days did not have significant effects on the humoral immune response, macrophage function, or cell-mediated immune response. There were also no changes in organ weights.	71
20-50 nm anatase TiO_2 NP	Oral administration of 10, 50, and 100 mg/kg/day TiO_2 NP for 14 days	Six-week-old male Swiss albino mice	Oral exposure to TiO_2 NP induced oxidative DNA damage in liver cells, possibly through oxidative stress mechanisms. This initiated the expression of apoptotic proteins, resulting in hepatic injury. Future studies need to investigate the safe use of TiO_2 NP.	72
46.23 nm 22% anatase/77% rutile TiO_2 NP	Oral administration of 5, 50, and 500 mg/kg/day TiO_2 NP for 5 days.	10-12 week-old male Swiss Webster mice	The orally administered TiO_2 NP were retained in the stomach for at least two weeks after the end of the five-day administration which led to chronic gastritis evidenced by dose-dependent p53-mediated apoptotic DNA damage, histopathological changes, and oxidative stress induction in gastric cells. Due to the accumulation in the stomach, even low TiO_2 doses (5 mg/kg in this study) can lead to development of gastritis.	73
Other models				
21 nm P25 Aeroxide TiO_2 NP (86% anatase/14% rutile) and 90 nm NM100 TiO_2 NP (98% anatase)	*C. elegans* were exposed to TiO_2 NP at 1, 3, 10, 30, and 100 mg/L for 96 hours, either in darkness or with solar irradiation.	*C. elegans*	Both TiO_2 samples were ingested by *C. elegans*, but the P25 NP were significantly more toxic than the NM100, and this difference seems to be related to particle size in addition to crystalline structure. TiO_2 NP toxicity was increased when particles were exposed to solar radiation (from a median effect concentration of > 100 mg/L to 53 mg/L), but the photocatalytic activity did not increase oxidative stress in *C. elegans* tissue. However, the intestinal cells would be directly	74

			exposed to any ROS produced by the NP and thus the intestines are a likely site of inhibitory processes that determine the phototoxic effect of the TiO_2 NP.	
5, 10, 60, and 90 nm TiO_2 NP	Exposure to 0.001, 0.01, 0.1, 1, 10, and 100 µg/L, and 1, 10, and 50 mg/L TiO_2 NP mixed with food for 8 days	*Caenorhabditis elegans*	More severe adverse effects were observed in *C. elegans* nematodes exposed to the smaller TiO_2 NP (range of ng/L, likely environmentally relevant doses) versus the larger TiO_2 NP (range of µg/L). Changes in locomotion behavior were found to be highly correlated to ROS production and both could be inhibited by antioxidant treatment. Thus, particle characteristics likely play an important role in NP toxicity.	75
10 nm anatase TiO_2 NP	Exposure with food to 1, 10, and 100 µg/L, and 10, 50, and 100 mg/L TiO_2 NP for 24 or 96 hours.	*C. elegans*	This study investigated recovery responses to TiO_2 NP exposure and found that prolonged exposure to 100 µg/L TiO_2 NP resulted in the inability of animals to successfully recover and return to a normal healthy state. Instead, nematodes exhibited severe deficits in intestinal barrier development and neurons controlling defecation. Thus, the intestinal barrier and defecation behavior may play a significant role in recovery after TiO_2 NP exposure.	76
10-30 nm rutile TiO_2 NP	Fish were exposed to 10 or 100 mg/L TiO_2 NP for 5 days.	Goldfish (*Carassius auratus*)	Short-term exposure to 10 or 100 mg/L TiO_2 NP was not lethal to goldfish. At both doses, Ti accumulated in gills and intestines, but not within muscle or brain. At the higher dose, goldfish exhibited weight loss and inhibition of growth as well as lipid oxidation as measured by detection of malondialdehyde in the liver. The potential for adverse effects of TiO_2 NP on aquatic organisms needs to be further investigated in longer-term studies with more realistic doses.	77
47 nm uncoated and 5 nm sulfur-doped TiO_2 NP	Zebrafish embryos were exposed to 10-1000 ppm TiO_2 NP in water for 96 hours. Some embryos were simultaneously exposed to solar	Zebrafish	Little toxicity was observed for uncoated TiO_2 NP, but the S-TiO_2 NP displayed toxicity which was increased by exposure to simulated sunlight irradiation to an LC_{50} of 116.56 ppm. S-TiO_2 NP exposure caused damaged to hair cells in neuromasts of the posterior lateral line which induced dysfunction in rheotaxis, likely through the observed ROS production and DNA damage. Although larvae were able to eliminate some of	78

	simulated sunlight.		the NP, TiO_2 remained in vital organs including the yolk sac, pneumatic islet, and heart, and were localized within cells in small aggregates. NP seemed to be internalized by lysosome-like vesicles. S-TiO_2 NP were able to induce cell death, partially through formation of giant vacuoles in cells.	
44.1 nm TiO_2 and 43.0 nm $AgTiO_2$ NP	Exposure in water containing 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 10, 50, and 100 mg/L TiO_2 NP and 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 5, 10, 20, and 40 mg/L $AgTiO_2$ NP from 24-96 hours.	*Artemia salina* nauplii	The LC_{50} value of TiO_2 NP exposure to nauplii was 381.6 mg/L after 24-hour exposure and 18.77 mg/L after 96-hour exposure. The $AgTiO_2$ NP were more toxic, with LC_{50} values of 23.03 mg/L after 24-hour exposure and 0.79 mg/L after 96-hour exposure. The exposed nauplii displayed enlarged intestines, eye shrinkage, paling of the eye, changes in the shape of the eye socket, and deformations of the outer shell. Based on this, release of TiO_2 or $AgTiO_2$ into aqueous environments can cause risks to aquatic organisms.	79

Table 5S: Summary of literature investigating ZnO nanoparticle toxicity *in vitro* since 2012.

ZnO NP characteristics	**Dose and treatment time**	**Cell type**	**Major conclusions**	**Ref.**
29.4 nm ZnO NP	10-100 mg/L, 24 hours	NCM460 (human colon mucosal epithelial cells)	Treatment of NCM460 cells with 100 mg/L ZnO NP caused rounding of cells and detachment from plates indicative of apoptosis. There was an ~70% decrease in viability of cells after 24-hour treatment with 100 mg/L ZnO based on PI staining, and Annexin V/PI staining showed	36

			that the majority of cell death was due to apoptosis. At the non-cytotoxic dose of 10 mg/L ZnO treatment for 24 hours, ROS levels were raised by 1.7-fold, as measured using DCFH-DA, suggesting that oxidative stress is contributing to the cytotoxic mechanism.	
20 nm ZnO NP	0.1-100 mg/L, 4-48 hours	Caco-2 and SW480 cells	ZnO NP were toxic, as evidenced by significant cell death beginning at 1 mg/L in SW480 cells with buffered synthetic freshwater and 10 mg/L in Caco-2 cells and SW480 cells with cell culture media and induced IL-8 generation, although there was no increase in ROS production. The inflammatory response (IL-8 generation) may indicate oxidative stress. Cell death was greater in buffered synthetic freshwater than in cell culture media or buffered synthetic freshwater with added organic material. Differences between Caco-2 and SW480 cells suggest that not all intestinal epithelial cells may react the same to ZnO NP.	37
Uncoated 100 nm ZnO NP (NM110)	2-128 μg/mL, 24 hours	Caco-2 cells	Exposure of cells to both ZnO NP (16 μg/mL) and palmitic acid enhanced cytotoxicity, likely through the induction of mitochondrial ROS production. However, addition of free fatty acids did not affect ROS production. Both ZnO NP and the fatty acids induced lysosomal destabilization but this was not correlated with cytotoxicity. The synergistic effects between ZnO NP and fatty acids should be considered further when evaluating NP toxicity.	80
10 nm, 36 nm, 389 nm, and 10 μm ZnO particles	0.125-4 mg/mL ZnO NP, 12-48 hours	Caco-2 cells	Only the nanoparticulate ZnO was able to induce toxicity in Caco-2 cells at concentrations above 0.125 g/L. The 389 nm or 10 μm ZnO particles did not cause toxicity even at the highest doses. Thus, larger ZnO particles may be better for antimicrobial purposes without causing toxicity to mammalian cells.	81
50-70 nm ZnO NP	1-20 $\mu g/cm^2$, 6-24 hours	Caco-2 cells	ZnO NP increased intracellular ROS levels after 6-hour exposure to Caco-2 cells at 1 $\mu g/cm^2$ and also increased IL-8 release, suggesting a role of oxidative stress in the ZnO NP response. The accumulation of ROS and induction of oxidative stress may lead to the observed cytotoxicity, but further studies are needed to fully understand this mechanism. The cytotoxicity of ZnO NP seemed to be related to ion dissolution and was greater in serum-free media.	39
26.21 nm	6.25-100	Caco-2 cells	ZnO NP exposure to Caco-2 cells induced dose-dependent oxidative	82

spherical, 62.42 nm rod-shaped, and 90.81 nm rod-shaped ZnO NP	μg/mL, 12-36 hours		stress, decreased cell viability, and increased cell death beginning at 6.25 μg/mL. The smallest (26 nm) ZnO NP had the highest toxicity. All sizes of ZnO NP caused a significant reduction in GSH with an increase in ROS and LDH release. The 26 nm ZnO NP also caused cell cycle inhibition, as evidenced by a decrease in G1 phase cells and an increase in S and G2 phase cells in order to repair damaged genes. Further studies are needed to understand the size-dependent behavior of ZnO NP in cells.	
10-15 nm ZnO NP	25-100 μM, 4 hours	IEC-6 cells	Treatment of IEC-6 cells with 25 μM ZnO NP was able to protect cells against H_2O_2-induced apoptosis. However, treatment of cells with 50 or 100 μM ZnO NP added to the toxicity of H_2O_2 in inducing apoptosis. Both ZnO NP and $ZnSO_4$ were more cytotoxic than bulk ZnO, and bulk ZnO also did not display protective effects at low dose. The cytotoxicity was at least partially mediated by an increase in Bcl-2 gene expression and inhibition of caspase-3, caspase-8, and caspase-9 gene expression.	83
10-20 nm spherical and 5-10 × 50-200 nm rod-like ZnO NP	10 μg/cm^2, 24 hours	C2BBe1 cells	ZnO NP increased LDH release and decreased metabolic activity in cells at 10 μg/cm^2. However, when ZnO NP were exposed to *in vitro* digestive solutions, the ZnO dissolved in the solution of pepsin at pH 2 representing stomach. Therefore, the toxicity of ZnO NP to intestinal epithelial cells may not be relevant if they do not make it past the stomach as NP.	7
10-20 nm spherical and 5-10 × 50-200 nm rod-like ZnO NP	1-10 μg/cm^2, 24 hours-10 days	C2BBe1 cells	Treatment of proliferating cells with ZnO NP completely inhibited cell proliferation at 10 μg/cm^2. However, it is unclear if this would be a problem at realistic oral exposure doses, so further studies are needed to investigate the relevance *in vivo*.	8
90 nm ZnO NP	10-200 μg/mL, 24 hours	Caco-2 cells	ZnO NP induced dose-dependent cytotoxicity at a dose of 10 μg/mL. ZnO NP treatment increased intracellular ROS and GSH levels and decreased SOD levels beginning at 10 μg/mL, indicating oxidative stress. Further studies are needed to determine *in vivo* toxicity.	84
50-70 nm ZnO NP	1-20 μg/cm^2 (6.4-128 μg/mL), 6-24	Caco-2 cells	ZnO NP exposure to Caco-2 cells increased 8-oxodG levels in cells (at 2.5 μg/cm^2) and induced formation of micronuclei (at 3.5 μg/cm^2) and DNA damage (beginning at 1 μg/cm^2). Cells exposed to 2.5 μg/cm^2	44

	hours		ZnO NP were unable to repair the oxidative DNA damage, revealing significant cytotoxicity affecting repair pathways in addition to inducing DNA damage.	
30 nm ZnO NP	10-100 µg/mL, 12-36 hours	Caco-2 cells	A dose- and time-dependent ZnO NP toxicity was observed with toxicity beginning at 10 µg/mL. ZnO NP depleted SOD and increased ROS and GSH levels, suggesting that toxicity may be mediated through oxidative stress. Further research is needed to assess the safety of ZnO NP for use in food packaging.	85
< 10 nm ZnO NP	0.3125-80 µg/cm^2, 1-24 hours	Caco-2 cells, undifferentiated and differentiated	ZnO NP induced toxicity and inflammation in Caco-2 cells, and undifferentiated cells were much more sensitive to this toxicity (significant toxicity at 5 µg/cm^2 after 24-hour exposure in undifferentiated cells vs. 20 µg/cm^2 in differentiated cells). However, the differentiated cells more closely model the intestinal epithelium, so evaluation of ZnO NP toxicity to them is needed as well. Simulated gastrointestinal digestion of the ZnO NP prior to cell exposure had a negligible impact on NP toxicity.	5
25.8 nm spherical ZnO NP	62.5-1000 µM, 3-24 hours	SW480, DLD-1, and NCM460 cells	ZnO NP exposure induced an inflammatory response, oxidative stress, cell cycle arrest, and cell death in intestinal cells at 250-1000 µg/mL. This toxicity was observed in the different intestinal cell types used to a different degree, likely due to the contrasting genetic landscape among cell lines. Thus, the cell model is as important as NP characteristics in determining the observed toxicity.	17
25.8 nm ZnO NP	6.25-250 µM, 5-24 hours	SW480, DLD-1, HCT116, and NCM460 cells	Different p53 status between the colorectal cell lines determined the oxidative stress response and survival of ZnO NP-treated cells (200 µM) where the cell lines with mutated p53 (DLD-1 and SW480) produced more ROS and had greater cell death in comparison to NCM460 and HCT116 cells with functional p53, revealing that p53 is able to regulate the antioxidant defense mechanism to protect cells from ZnO NP.	86

Table 6S: Summary of *in vivo* chronic oral ZnO nanoparticle exposure studies since 2012.

ZnO NP characteristics	**Dose and duration of exposure**	**Animal model**	**Major conclusions**	**Ref.**
Rat models				
< 100 nm ZnO NP	Oral administration of 10 mg/kg ZnO NP for 30 days. Rats were administered a single intraperitoneal dose of streptozotocin to induce diabetes prior to treating with ZnO NP.	Six-month-old male Sprague-Dawley rats	ZnO NP were found to be antidiabetic agents. Blood glucose levels were decreased by 75.8% after treatment with ZnO NP and serum insulin was increased by 79.4%. There was an increase measured in expression of insulin receptors and GLUT-2 as well as glucokinase activity (52.5%) in the liver of treated rats compared to untreated diabetic rats.	87
Hexagonal 26.4 ± 6.1 nm ZnO NP	For dosing purposes, rats were administered 536.8, 1073.5, and 2147 mg/kg/day ZnO NP for 14 days. Rats were treated with 134.2, 268.4, and 536.8 mg/kg/day ZnO NP for 13 weeks.	Seven-week-old Sprague-Dawley rats	After 13-week 536.8 mg/kg ZnO NP administration, Zn accumulation was observed in blood, liver, kidney, and urine, but the absorption was low. Further study is needed to determine the effect of physicochemical properties of NP on determining absorption kinetics. The effect of ZnO dissolution in gastric fluid should also be investigated.	58
20 nm ZnO NP, suspended in citrate and HEPES for administration	Rats were administered ZnO NP via oral gavage at doses of 125, 250, and 500 mg/kg/day for 90 days.	Six-week-old Sprague-Dawley rats	Oral administration of up to 500 mg/kg ZnO NP did not cause mortality or a change in body weight, but there were some abnormal symptoms including piloerection and alopecia. After 90-day administration, there were slightly increased levels of ZnO in the groups treated with 125 mg/kg, and significant accumulation was observed at 250 and 500 mg/kg doses. There was no significant difference between male and female rats. Therefore, ZnO NP could accumulate *in vivo* in systemic circulation at a dose of at least 125 mg/kg, and the oral NOAEL would be less than 125 mg/kg.	88
50 nm ZnO NP	Oral administration of	Wistar albino	ZnO NP oral exposure (600 mg/kg) increased serum	89

	600 or 1000 mg/kg/day for 5 days. Some rats were also administered α-lipoic acid or vitamin E daily for 3 weeks starting at the same time as ZnO administration.	rats	cardiac injury markers in rats including troponin-T, creatine kinase-MB, and myoglobin. Pro-inflammatory biomarkers including TNF-α, IL-6, and C-reactive protein were increased after ZnO NP administration, and NO and VEGF levels were increased. Cardiac calcium concentration, oxidative DNA damage, and caspase-3 activity increased after ZnO NP exposure. Co-administration of ZnO NP with vitamin E or α-lipoic acid significantly ameliorated the cardiotoxicity of the NP, and thus may be used to protect against ZnO NP toxicity.	
20-30 nm ZnO NP	Oral administration of 333.33 mg/kg/day ZnO NP for 5 days.	Six-eight-week-old female Wistar rats	Oral administration of 333.33 mg/kg ZnO NP resulted in symptoms of vomiting, loss of appetite, and severe lethargy in rats, demonstrating severe toxicity within 5-day exposure. Lung, liver, and kidney were found to be target organs for ZnO NP. Therefore, precautions should be taken when handling ZnO NP.	90
20 nm ZnO NP, capped with L-serine	Oral administration of 100, 200, or 400 mg/kg/day to pregnant rats from gestational days 5 through 19.	12-week-old Sprague Dawley rats	Administration of ZnO NP to pregnant rats had minimal impact on intrauterine fetal growth and development up to a dose of 400 mg/kg/day, and 200 mg/kg/day was considered the NOAEL for both maternal toxicity and embryo-fetal development. Decreased body weight and liver and adrenal gland weight was observed in dams at the 400 mg/kg/day dose and reduced food consumption was observed at both 200 and 400 mg/kg/day. Some differences were observed in fetal weights and increased abnormalities were also observed after administration of 400 mg/kg/day ZnO NP. Therefore, there still needs to be further research on the effects of ZnO NP on reproduction and development.	91
35 nm ZnO NP	Oral administration of 500 mg/kg/day for 6 weeks to male rats beginning 2 weeks	Sprague Dawley rats	ZnO NP administration was found to be able to transfer and accumulate over the lactation and placental barriers and induce reproductive developmental toxicity. Fertility and mating parameters were unaffected by 500 mg/kg	92

	prior to mating and to female rats from 2 weeks before mating through the gestation period to day 4 of lactation.		ZnO exposure, but developmental toxicity was observed in the offspring of ZnO NP-exposed dams including a reduced number of pups born and live pups, decreased body weights of pups, and increased fetal resorption. ZnO NP were distributed to tissues including mammary tissue of dams and liver and kidney of pups. Further study is needed to determine whether there is a risk for humans.	
100 nm ZnO NP, coated with citrate to create a negative charge and L-serine for a positive charge	Oral administration of 500, 1000, and 2000 mg/kg/day for 14 days, and of 31.25, 125, and 500 mg/kg/day for 90 days.	Sprague Dawley Crl:CD(SD) rats	ZnO NP 90-day oral administration induced symptoms in rats including salivation, white feces, changes in water and feed consumption, and hematological changes. There were also histopathological changes including squamous cell hyperplasia and vacuolation in nonglandular stomach, inflammatory cell infiltration and mucus cell hyperplasia in the glandular stomach, chronic inflammation and acinar cell apoptosis in the pancreas, suppurative inflammation in the prostate gland, and retinal atrophy in the eye. The target organs for ZnO NP were considered to be the stomach, pancreas, eye, and prostate gland, and significant toxicity was observed in males and females at doses above 125 mg/kg, and the NOAEL was considered to be 31.25 mg/kg for both the positively and negatively charged ZnO NP.	93
29 nm ZnO NP, coated with citrate to create a negative charge	Oral administration of 125, 250, and 500 mg/kg/day for 90 days.	Six-week-old Sprague Dawley rats	No NOAEL was determined from the administration of 125, 250, and 500 mg/kg/day to rats for 90 days, but the lowest-observed-adverse-effect-level (LOAEL) was determined to be 125 mg/kg. Symptoms, particularly at 500 mg/kg included increased salivation, acinar cell apoptosis, chronic inflammation of the pancreas, inflammatory stomach lesions, retinal atrophy of the eye, and changes in anemia-related parameters. Thus, ZnO NP should be used with caution in human medicine.	94
40 nm ZnO NP	Oral administration of	Sprague	Male rats administered 536.8 mg/kg for 13 weeks had	95

	536.75, 1073.5, or 2147 mg/kg/day ZnO NP for 14 days, and 67.1, 134.2, 268.4, or 536.8 mg/kg/day for 13 weeks.	Dawley rats	lower body weight gain than control rats. Both male and female rats receiving 536.8 mg/kg displayed changes in anemia-related parameters, and mild to moderate pancreatitis. The NOAEL was determined to be 268.4 mg/kg/day, which suggests that ZnO NP are safe for use in foods and food supplements.	
20 and 100 nm ZnO NP, coated with citrate to create a negative charge and L-serine to create a positive charge	Oral administration of 500 mg/kg/day ZnO NP for 28 days.	Six-week-old rats	After the 28-day oral administration of 500 mg/kg ZnO NP, the blood brain barrier was found to be intact, suggesting no significant damage. Thus, this study suggests no significant neurotoxicity of ZnO NP to the brain.	24
20 nm ZnO NP	Oral administration of 100, 200, and 400 mg/kg/day for 14 days.	Eight-week-old female Wistar rats	Oral administration of 400 mg/kg ZnO NP to rats was able to cause damage to lung tissue and hepatocytes and elevate levels of LDH. Increasing doses of ZnO NP caused damage to lung tissue and increased LDH, IgG, TNF-α, and IL-6. Thus, exposure to high concentrations of ZnO NP could cause irreversible damage to organs including lungs, and potential health hazards of ZnO must be taken into consideration.	96
10-15 nm ZnO NP	Oral administration of 1, 3, 10, 30, and 300 mg/kg ZnO NP for 28 days.	Eight-week-old Wistar rats. Type 1 diabetes was induced by injecting streptozotocin into the tail vein of adults. Type 2 diabetes was induced by IP	ZnO NP oral administration was able to elicit antidiabetic activity including improved glucose tolerance, higher serum insulin, reduced blood glucose, reduced nonesterified fatty acids, and reduced triglycerides in rat models of Type 1 and Type 2 diabetes. ZnO NP also enhanced SOD and catalase activities in rats. Higher zinc levels were observed in liver, adipose tissue, and pancreas. ZnO NP were observed to be safe up to a dose of 300 mg/kg in rats. Thus, ZnO NP should be further investigated as potential antidiabetic agents.	97

		injection of streptozotocin in 5-day-old pups.		
< 100 nm ZnO NP and bulk ZnO	Oral administration of 500 mg/kg/day ZnO NP and bulk ZnO for 10 days. Some rats were also co-administered B vitamins.	Male albino rats	The co-administration of B vitamins with 500 mg/kg ZnO NP helped to attenuate inflammation (including TNF-α and C-reactive protein upregulation) and apoptotic oxidative DNA damage (including VEGF and malondialdehyde expression) induced in rat kidneys by ZnO NP or bulk ZnO. Thus, prophylactic treatment with B vitamins may help protect the kidney from ZnO toxicity.	98
< 100 nm ZnO NP and bulk ZnO	Oral administration of 500 mg/kg/day ZnO NP or bulk ZnO for 10 days. B vitamins were co-administered to some rats.	Male albino rats	Prophylactic supplementation of vitamin B complex was beneficial against inflammation and apoptotic oxidative DNA damage induced in rat livers by 500 mg/kg ZnO NP or bulk ZnO. ZnO administration increased serum ALT, AST, LDH, glucose, TNF-α, and C-reactive protein, as well as VEGF and liver oxidative DNA damage and the apoptosis marker caspase 3 in the liver, all of which were ameliorated with vitamin B complex administration. Thus, early treatment with vitamin B complex should be considered to protect the liver from ZnO-induced damage.	99
20-30 nm ZnO NP	Oral administration of 100, 200, and 400 mg/kg/day ZnO NP for 10 days.	Eight-week-old male Wistar rats	Oral administration of 400 mg/kg ZnO NP to rats were found to increase serum levels of the tumor markers prostate specific antigen and carcinoembryonic antigen, possibly due to increased free radical levels inducing apoptosis. ZnO NP also increased 1-α-fetoprotein, indicating liver damage.	100
20 nm hexagonal ZnO NP	Oral administration of 40, 70, 100, or 150 mg ZnO NP/mL/day for 8 weeks.	75-90-day-old male Wistar rats	After ZnO NP administration, dose-dependent atrophy and degeneration of acini and striated ducts dilatation in the submandibular gland were observed beginning at 40 mg/mL along with heterogenic chromatin condensation,	101

			degenerated mitochondria, and few zymogen granules.	
ZnO NP (no size given)	Oral administration of 10 mg/kg/day ZnO NP for 5 days.	Male Wistar rats	Exposure of rats to 10 mg/kg ZnO for 5 days decreased AST and ALT levels and caused minor morphological changes in liver, kidney, and brain. No behavioral changes were observed.	102
20 nm citrate-capped ZnO NP	Oral administration of 100, 200, or 400 mg/kg/day ZnO NP from gestational days 5-19.	Ten-week-old Sprague Dawley rats	Based on this administration of ZnO NP to pregnant rats, no embryo-fetal developmental toxicity was apparent and 400 mg/kg/day was considered the NOAEL for embryo-fetal development. In the mothers, a decrease in body weight was observed at 400 mg/kg and decreased liver weight and increased adrenal gland weight were observed at 200 mg/kg, and thus the maternal NOAEL was considered to be 100 mg/kg/day.	103
50 nm ZnO NP	Oral administration of 600 or 1000 mg/kg/day ZnO NP for 5 days. Some groups of rats at each dose were administered 200 mg/kg/day L-arginine, quercetin, or both in addition to ZnO NP.	Male Wistar albino rats	Hepatotoxicity was observed after 600 mg/kg ZnO NP administration, as evidenced by elevated inflammatory cytokines and increased serum ALT and glucose. ZnO NP also induced oxidative DNA damage in liver cells. The co-administration of ZnO NP with L-arginine, quercetin, or both all reduced the DNA damage and the upregulation of the observed biomarkers, possibly due to attenuation of ROS production and inflammation.	104
< 100 nm ZnO NP	Oral administration of 40 and 100 mg/kg/day ZnO NP for 1 week.	Adult male Wistar albino rats	Oral exposure of rats to 40 mg/kg ZnO NP induced significant toxicity to the lung including an inflammatory response, DNA damage, and oxidative damage as evidenced by increased lipid peroxidation and decreased GSH, CAT, and PON-1.	105
29 nm serine-modified ZnO NP (positively charged)	Oral administration of 125, 250, and 500 mg/kg/day ZnO NP for 90 days.	Six-week-old Sprague Dawley rats	Administration of 125 mg/kg ZnO for 90 days to rats caused adverse effects to the pancreas (acinar cell apoptosis), stomach (submucosal edema and inflammation), and eye (retinal atrophy in the 500 mg/kg group). The LOAEL was considered to be < 125 mg/kg, and target organs were the stomach, pancreas, and eye.	106

Mouse models				
20 and 100 nm ZnO NP, coated with citrate to make particles negatively charged and with L-serine to make them positively charged	Oral administration of 750 mg/kg/day for 14 days.	Six-week-old C57BL/6 mice	ZnO NP oral administration induced immunotoxicity, which was observed as minor immunosuppression. This included suppression of NK cell activity, a slight reduction in the $CD4^+/CD8^+$ ratio, lower nitric oxide production from splenocytes of 750 mg/kg ZnO NP-fed mice, and suppression of inflammatory cytokines. This may be problematic for those who are chronically exposed to ZnO NP.	107
30 nm ZnO NP	Oral administration of 50 or 300 mg/kg ZnO NP for 14 days	Six-week-old male Swiss albino mice	Exposure of mice to 300 mg/kg ZnO NP for 14 days resulted in accumulation of NP in the liver and induction of oxidative stress leading to DNA damage and subsequent apoptosis in liver cells. This suggests the need for a complete risk assessment of all new NP before their use in the consumer market.	108
80-100 nm ZnO NP	Oral administration of 500 mg/kg/day ZnO NP for 21 days.	Seven-week-old male *Swiss albino* mice	Oral administration of 500 mg/kg ZnO NP to mice for 21 days resulted in generation of ROS, leading to cellular toxicity if the magnitude of ROS production overwhelmed the antioxidant defense status of the cell. Accumulation of the ZnO NP in the brain disturbed the normal metabolism of neurotransmitters, leading to brain damage and indicating the neurotoxic potential of the NP. Inhibition of CuZnSOD and MnSOD in the liver and brain further established oxidative stress as the major mechanism of toxicity. Within cells, ZnO NP were found within the cytoplasm, mitochondria, and nucleus.	70
ZnO NP (no size given)	Oral exposure to 100 or 300 mg/kg ZnO NP in drinking water for 28 days.	One-month-old female NMRI mice	After administration of ZnO NP in drinking water to mice, there was an observed NP accumulation in the heart which was significant at 300 mg/kg. Thus, potential ZnO NP toxicity to the heart should be investigated further.	109
15-20 nm ZnO NP	Oral administration of 10 mg/kg/day ZnO NP	Nine-eleven-week-old	ZnO NP (10 mg/kg) exhibited anticoccidial activity in infected mice, as evidenced by a significant decrease of	110

	for 5 days to mice which had been orally inoculated with *E. papillata*.	C57BL/6 mice	the oocysts in feces and a decrease in symptoms of infection including inflammation, nitric oxide production, malondialdehyde, and increase in GSH. Thus, ZnO could be used for its protective effects.	
80 nm ZnO NP	Oral administration of 200 or 400 mg/kg/day ZnO NP for 90 days.	Six-week-old male C57BL/6 mice	ZnO NP administration (200 mg/kg) induced liver injury due to oxidative stress and ER stress including ER stress-mediated apoptosis.	111
Six ZnO NP samples (hydrodynamic diameters of 149, 121, 112, 112, 125, and 133 nm)	Oral administration of 14 mg/kg ZnO NP twice daily for 90 days. Some mice were also administered 50 mg/kg vitamin C twice daily.	Three-four-week-old or five-six-week-old male Kunming mice	Vitamin C increased the toxicity induced by 14 mg/kg ZnO NP administration which may be due to an acceleration of dissolution. Liver and kidney damage was greater after administration of vitamin C and ZnO NP than either alone. This highlights the importance of the complex system within nanofoods which will determine toxicity.	112
Other models				
10, 50, and 100 nm ZnO NP	Exposure to 0.1-2.0 g/L for 24-48 hours	*C. elegans*	The *C. elegans* activity was not adversely affected after exposure to 50 or 100 nm ZnO NP at up to 1 g/L, but the 10 nm ZnO NP increased *mtl-1* and *sod-1* gene expression at 0.7 g/L. The intestine was the major target organ of ZnO NP, and the 10 nm ZnO NP were the most toxic. Further studies are needed to determine kinetics of distribution and dose-response.	113
Uncoated 16-50 nm ZnO NP	*Artemia salina* larvae were exposed to 10 and 100 µg/mL suspensions of ZnO NP for 24 hours. Goldfish were exposed to 1 and 10 µg/mL suspensions of ZnO NP for 21 days and the dietary exposure group was fed	*Artemia salina* and goldfish (*Carassius auratus*)	The *Artemia* treated for 24 hours with 10 µg/mL ZnO were found to contain 255 ± 35 µg/g Zn while the *Artemia* treated with 100 µg/mL ZnO NP were found to contain 705 ± 45 µg/g Zn. For both waterborne and dietary ZnO NP exposure, Zn primarily accumulated in goldfish intestine, followed by the gills and liver, with very little Zn detected in heart, brain, and muscle. Zn concentrations were significantly greater in tissues after waterborne exposure than dietary exposure (~2, 4, and 10-fold higher in liver, gills, and intestine, respectively).	114

	ZnO-exposed *Artemia* at amounts designed to create concentrations of 1 and 10 µg/mL ZnO.		No increase in malondialdehyde levels was detected, suggesting little ZnO toxicity to goldfish.	
50-150 nm spherical ZnO NP and ellipsoidal bulk ZnO	Exposure to 2 mg/L in water for 30 days.	Goldfish (*Carassius auratus*)	The ZnO NP and bulk ZnO aggregated in the gills and gut and thus Zn was not transported to internal tissues. Metal-rich granules were the main mechanism of liver detoxification after ZnO NP exposure while metallothionein-like proteins were the main target of Zn^{2+}, which localized to cell organelles. Thus, subcellular partitioning may be important in the mechanism of NP toxicity.	115
38.3 and 74.8 nm ZnO NP	*Xenopus laevis* embryos were exposed to 100 mg/L ZnO NP suspensions for 96 or 120 hours post-fertilization.	*Xenopus laevis*	Upon ZnO NP exposure, NP induced malformations and histological lesions to the gut, likely due to oxidative damage. ZnO NP also disrupted the cytoskeletal organization and ZO-1 scaffold proteins of the cellular junction complex, leading to a loss of barrier integrity. ZnO NP also induced cytotoxicity, with the smaller NP causing stronger effects than the larger NP. Thus, further studies must be done to determine how to limit ZnO NP toxicity.	116
Uncoated, polyvinylpyrrolidone-, or polyethylene glycol-coated 63 ± 29 nm round and 334 ± 208 nm rod-shaped ZnO NP	*Xenopus laevis* embryos were exposed to ZnO NP at concentrations of 1, 10, 50, and 100 mg/L for up to 96 hours post-fertilization.	*Xenopus laevis* embryos	All ZnO NP were toxic to *Xenopus laevis* embryos, causing malformations, and the gut is the most sensitive organ. The smaller, round ZnO NP were more effective than the larger, rod-shaped ZnO NP, and PEGylation also reduced the NP toxicity. Thus, further research needs to be done to determine whether certain properties can make ZnO NP safe.	117
21 nm and 35 nm ZnO NP	Exposure to 0.1, 0.33, 1, 3.3, and 10 mg/L ZnO NP for 24-48 hours.	*Daphnia magna*	The smaller ZnO NP were found to be more toxic to *D. magna* than the larger ZnO NP. This may be because 10-30 nm was determined to be the optimal size for NP to gain access to most of the cellular compartments, and the smaller NP had a greater proportion of particles within	118

			this 10-30 nm size range. The ZnO NP toxicity was found to be independent of dissolved Zn ions in the media. ZnO NP were readily internalized by intestinal epithelial cells and were localized to a wide range of intracellular compartments, indicating that the degeneration of the digestive epithelium is the primary toxicity response *in vivo*. ZnO NP were internalized by cells either by penetrating the cell without membrane disruption or by endocytic pathways. Internalized ZnO NP were able to cause vacuolization of the cytoplasmatic matrix, mitochondrial dissolution, and formation of degenerative/defensive structures such as multilamellar bodies and multivesicular bodies. NP were also present in gut muscularis and some cells of the hemocoel, suggesting that they can cross the epithelial barriers. More information is needed to determine toxicity after longer exposures.	
30 nm ZnO NP	Administration of 0.5, 5, and 50 mg/L ZnO NP for 14 days.	Carp (*Cyprinus carpio*)	ZnO NP exposure induced toxicity but was not lethal to carp at 0.5-50 mg/L, and toxicity was more significant with increasing dose and treatment time. ZnO NP-induced oxidative stress led to depletion of antioxidant enzyme activities and elevation of lipid peroxidation levels at 50 mg/L ZnO NP. Susceptibility to the toxicity varied depending on organ; gills, liver, and brain were the more sensitive organs and intestine was the least sensitive. More studies are needed to evaluate the effects of ZnO NP on aquatic ecosystems.	119
30 nm spherical ZnO NP and 2 μm bulk ZnO	Exposure to 50 mg/L ZnO NP and bulk ZnO in water for 30 days.	Carp (*Cyprinus carpio*)	ZnO NP accumulated in carp and caused higher levels of intracellular oxidative stress and more severe histopathological changes than the bulk ZnO after 30-day exposure at 50 mg/L. Liver and gill appeared to be the target tissues for ZnO NP. Zn^{2+} toxicity was determined to be negligible in these conditions, and thus the toxicity	120

			was attributed to particle size. Further study is needed to determine effects of environmental parameters on ZnO NP toxicity and the mechanism of toxicity.	

Table 7S: Summary of *in vitro* Ag nanoparticle toxicity studies since 2012.

Ag NP characteristics	**Dose and treatment time**	**Cell type**	**Major findings**	**Ref.**
15 nm AgPURE NP (in solution as 10% silver stabilized with 4% Tagat TO and 4% Tween 20)	0.5-100 µg/ml, 24 hours	Caco-2 cells, proliferating and differentiated	Significant acute toxicity after 24-hour Ag NP exposure in differentiated cells beginning at 25 µg/ml without serum and 50 µg/ml with serum based on the CellTiter Blue assay to assess viability and the DAPI assay to assess cell proliferation. Toxicity was observed at 10 µg/ml in proliferating cells. The majority of Ag NP were not internalized into differentiated Caco-2 cells. Both Ag NP and Ag^+ released from the particles will interact with membrane-bound and extracellular proteins. Ag NP induced morphological changes and oxidative stress in cells and induced necrosis but no apoptosis. Necrotic cell death can trigger inflammation and may be especially problematic in those with inflammatory bowel disease.	121
20 nm Ag NP in citrate solution	0.01-20 µg/mL, 24 hours	Caco-2 cells	Treatment of Caco-2 cells with 20 nm Ag NP at 10 µg/mL induced cytotoxicity, DNA damage, and mitochondrial injury but no oxidative stress. This model may be useful for screening the cytotoxic potential of Ag NP.	122
20 nm Ag NP in citrate solution	1-40 µg/mL, 4-48 hours	Caco-2 cells	This study determined that Ag NP treatment may induce DNA damage, leading to genotoxicity. The flow cytometric micronucleus assay was used to detect DNA damage after treatment of cells with 15 µg/mL Ag NP, but the 1.5-fold increase in micronucleus frequency was not significant, suggesting that Caco-2 cells are not particularly susceptible to any Ag NP-induced genotoxicity.	123
Citrate-stabilized	1-15 µg/mL, 4-	Caco-2 cells	Ag NP were cytotoxic to Caco-2 cells based on resazurin (Alamar	124

20 nm Ag NP	24 hours		Blue) reduction and dsDNA content (reported in Sahu et al. 2014).[122, 123] Using the cytochalasin B-blocked micronucleus assay, it was observed that the micronucleus frequency increased from a background level of 1.4-1.9% to 2.7-3.4% in Caco-2 cells treated with 20 nm Ag NP for 4-24 hours. The increase in micronucleated cells was significant at 10 and 15 µg/mL after 4-hour treatment and at 15 µg/mL after 24-hour treatment, suggesting that Ag NP can induce genotoxicity in Caco-2 cells, potentially via an apoptosis pathway independent of ROS and dependent on mitochondrial protein alterations.	
< 100 nm Ag NP	5-1000 µg/mL, 24 hours	Caco-2 cells	The IC_{50} for Ag NP in Caco-2 cells was determined to be 16.7 µg/mL based on the MTT assay and 14.9 µg/mL based on the Trypan blue exclusion assay. No ROS production was detected by the DCF or Mitotracker assays, but GSH levels were significantly decreased after 24-hour Ag NP treatment beginning at 5 µg/mL. There was also a dose-dependent depolarization of the mitochondrial membrane potential in response to Ag NP treatment. Ag NP treatment also activated the stress-responsive gene Nrf2 and increased expression of HO-1, downstream of Nrf2. Thus, Ag NP are able to induce acute cytotoxicity by an oxidative stress-related pathway in Caco-2 cells.	125
25 nm spherical and 80-90 nm rod-shaped Ag NP	1-243 µg/mL, 4-48 hours	Caco-2 cells	Treatment of cells with 9 µg/mL Ag NP for 48 hours caused death of cells at the edges of cell clusters, and 27 µg/mL Ag NP treatment induced cell necrosis. A decrease in enzymatic activity was observed at 81 µg/mL Ag NP treatment. Cells internalized Ag NP (27 µg/mL dose for 48 hours) without causing changes in cell morphology. No ROS production was observed after 4-hour treatment of cells with Ag NP at up to 243 µg/mL, but IL-8 secretion was detected after 48-hour treatment of cells with 27 µg/mL Ag NP. This study was done to assess the toxicity of NP-doped paint. No significant cytotoxic effects were observed for paint particles doped with Ag NP, but long-term studies of environmental NP exposure are still needed.	16
90 nm Ag NP	10-200 µg/mL, 24 hours	Caco-2 cells	No increase in cell death was observed based on acridine orange and ethidium bromide staining of 200 µg/mL Ag NP-treated cells. The	84

			CCK-8 viability assay revealed a decreased cell activity to ~65% at 25-200 μg/mL Ag NP. Treatment of cells with up to 200 μg/mL Ag NP for 24 hours did not induce ROS generation or alter the SOD level in cells, but GSH levels increased significantly after Ag NP treatment, most significantly at 10 μg/mL. Thus, these findings indicate little Ag NP cytotoxicity.	
5-45 nm bare and poly(α,γ L-glutamic acid) (PGA)-capped Ag NP	300 μL of the PGA-capped Ag NP solution (0.5 mL $AgNO_3$ in 31.5 mL total) in 800 μL total volume	Caco-2 cells	No changes in the number of viable cells were observed after treatment of cells with Ag NP (300 μL of the PGA-capped Ag NP). ZO-1 staining did not indicate tight junction damage although the staining pattern appears ruffled after Ag NP treatment. F-actin was partially redistributed to the cell membrane after Ag NP treatment, indicative of mechanochemical stress, potentially as a result of Ag NP internalization by cells. However, Ag NP were concluded to be biocompatible.	126
Uncoated 20-30 nm Ag NP	1-100 μg/mL, 24-48 hours	Caco-2 and SW480 cells	Ag NP were found to be largely non-toxic in Caco-2 and SW480 cells. The MTT assay revealed no significant changes in viability of cells treated with Ag NP for 24 or 48 hours although there was a dose-dependent trend towards decreased cell viability in Caco-2 cells which was not significant at up to 100 μg/mL. In buffered synthetic freshwater (to assess NP in drinking water), a similar dose-dependent trend towards decreased cell viability was observed in Caco-2 cells that was not significant up to 100 μg/mL, and significant toxicity was observed at 1.0 μg/mL in SW480 cells. No increase in ROS generation was observed, but significant IL-8 generation was induced after treatment with 10 μg/mL Ag NP for 48 hours. Thus, despite low toxicity of Ag NP, caution should be used to reduce human NP exposure.	37
30 nm Ag NP	10-100 μg/mL, 12-36 hours	Caco-2 cells	Ag NP displayed slight hormesis at concentrations ≤ 50 μg/mL and toxicity at doses ≥ 75 μg/mL. Ag NP dose-dependently depleted SOD levels and slightly increased ROS levels, but decreased GSH. Further research is needed to evaluate safety of Ag NP for use in food packaging.	85
< 20 nm	6.5 ppm, 15	Caco-2 cells	All Ag NP (conventional and “green”-coated) altered the membrane	127

uncoated, 5-10 nm glutathione-coated, and 5-20 nm green tea-coated Ag NP	minutes		permeability of Caco-2 cells based on TER after 15-minute exposure to 6.5 ppm. Further research is needed to determine if the coatings are unstable or if there are other coatings that could be used to make Ag NP safe for therapeutic uses.	
22 nm citrate-coated Ag NP, 19.4 nm citrate-coated Ag NP with Au cores, and 18.7 PVP-coated Ag NP with Au cores	0.1-50 μg/mL, 6-24 hours	Caco-2 cells	No toxicity of any of the Ag NP were observed up to 50 μg/mL although there was evidence of induction of oxidative stress based on increased HO-1 expression (but no change in GSH). Upregulation of metallothionein expression was observed which may contribute to the resistance of cells to Ag NP toxicity.	128
49.1 nm citrate-capped, 45.4 nm PVP-capped, and 49.8 nm tannic acid-capped Ag NP	1 μg/cm^2 (3 mg/L), 8-24 hours	Caco-2 cells	The internalization efficiency of Ag NP at 1 μg/cm^2 into Caco-2 cells was affected by the capping agent of the particles (tannic acid-capped Ag NP had the highest cellular uptake), but Ag NP did not induce cytotoxicity. This suggests that Ag NP may not induce adverse effects on cells at occupational and incidental exposure levels, and more studies should be done at relevant doses.	129
310-400 nm Ag NP synthesized with *Eclipta alba* leaf abstract	0.5-50 μM, 24 hours	Caco-2 cells	Ag NP dose-dependently decreased Caco-2 cell viability based on the MTT assay beginning at 0.5 μM.	130
25.8 nm Ag	1×10^{-12}-1 g/L, 24 hours	NCM460 (human colon mucosal epithelial cells)	There was a 17% decrease in viability of NCM460 cells treated for 24-hours with 1 g/L Ag NP based on PI staining. Although there was not significant cytotoxicity at drinking water-relevant concentrations, care must be taken to prevent persistence and accumulation of Ag NP in natural water resources.	36
Spherical citrate-stabilized 10, 20, 40, 60, and 100 nm Ag NP	1-10 μg/mL, 24 hours	LoVo human colon carcinoma cells	Ag NP treatment of cells for 24 hours significantly increased ROS generation in a size-dependent manner beginning at 5 μg/mL, with the greatest effects observed with smaller Ag NP. Induction of IL-8 release (beginning at 1 μg/mL), decreased mitochondrial activity of cells (10 μg/mL), and increased apoptosis and necrosis (10 μg/mL) all	131

			followed a size-dependent pattern. The decrease observed in cell proliferation was not size-dependent, and 10 μg/mL Ag NP diminished the proliferation rate to ~20% that of the untreated control. A proteomics study by mass spectrometry at a dose of 10 μg/mL of 20 or 100 nm Ag NP for 24 hours revealed downregulation of proteins involved in mitochondrial protein synthesis and upregulation of proteins involved in cellular redox regulation. More proteins involved in cell death and mitochondrial activity were affected by the 20 nm Ag NP while cell growth proteins were equally affected by 20 and 100 nm Ag NP. Cells were found to readily internalize the 20 nm Ag NP while rarely internalizing larger NP. This study suggests a significant role of size in determining NP toxicity, possible due to cellular internalization of NP.	
Citrate-stabilized 23 ± 8 nm Ag NP	0.25-10 μg/cm^2, 24 hours – 7 days	C2BBe1 cells, proliferating and confluent	In proliferating cells, 24-hour exposure to 0.25 μg/cm^2 pristine and 0.5 μg/cm^2 digested Ag NP induced 15-20% necrosis and a 76-86% reduction in metabolic activity, but no apoptosis. The GSH/GSSG ratio decreased after 24-hour treatment with 0.25 μg/cm^2 pristine Ag NP or 0.5 μg/cm^2 digested Ag NP, indicating induction of oxidative stress. A G2/M phase cell cycle arrest was observed in proliferating cells treated with Ag NP for 4 days, and a complete inhibition of cell proliferation was observed in growth curves. Ag NP toxicity appeared to be mediated through Ag^+. Ag NP were not toxic to confluent cells at 10 μg/cm^2 doses of Ag NP. Thus, NP toxicity should be particularly investigated in proliferating intestinal stem cells *in vivo*.	8
20 and 40 nm Ag NP coated with L-cysteine L-lysine L-lysine (CKK) peptide	5-100 μg/mL, 24-48 hours	Caco-2 cells, proliferating and differentiated	Treatment of Caco-2 cells with 5 μg/mL 20 nm Ag NP caused the rounding and detachment of cells from the culture plate. Impedance measurements showed an initial increase directly after treatment with both sizes of Ag NP, and then a dose-dependent decrease beginning at a dose of 5 μg/mL. The CellTiter-Blue viability assay showed acute toxicity of 20 nm Ag NP beginning at 5 μg/mL and of 40 nm Ag NP beginning at 50 μg/mL after 48 hours. Differentiated cells were much more resistant to Ag NP-induced toxicity. No apoptosis was observed in proliferating cells treated with 5 μg/mL of 20 or 40 nm Ag NP for	132

			up to 24 hours. A dose-dependent increase in ROS generation was detected by DCF assay after 24-hour treatment of proliferating cells with 20 and 40 nm Ag NP beginning at doses of 3 μg/mL 20 nm Ag NP and 10 μg/mL 40 nm Ag NP, which may be the mechanism of cytotoxicity.	
7.02 ± 0.68 nm Ag NP stabilized with Tagat TO and Tween 20	1-100 μg/mL, 24-48 hours	Caco-2 cells, proliferating and differentiated	Cytotoxicity of digested and non-digested Ag NP was detected beginning at 15 μg/mL by CellTiter-Blue assay and beginning at 7 μg/mL by DAPI staining in proliferating cells. Differentiated cells were less sensitive than proliferating cells. Impedance measurements showed that digested Ag NP required slightly higher doses (effects were observed at 5 μg/mL in non-digested and 10 μg/mL in digested Ag NP) and longer times (by ~12 hours) than the non-digested Ag NP to cause the same decrease in cell index. Although there was little change in cytotoxicity after digestion of Ag NP, the interference of the digestive process with NP characteristics should be considered when testing for toxicity.	133
35 nm Ag NP and 0.6-1.6 μm Ag microparticles	31.25 μg/cm^2, 6-24 hours	Differentiated Caco-2 cells	Ag NP treatment of Caco-2 cells for 6 or 24 hours showed uptake of NP by cells by confocal microscopy. Greater uptake of Ag NP was observed over Ag microparticles (5052 ± 1936 μm^3/mm^2 vs. 450 ± 255 μm^3/mm^2) after 24-hour exposure.	134
3.2 nm PAA-coated Ag NP	10-100 μg/mL Ag NP, 24 hours	Differentiated Caco-2 cells	Untreated Ag NP, Ag NP subjected to *in vitro* digestion, and Ag NP digested along with food components (milk powder, starch, and olive oil to represent proteins, carbohydrates, and fatty acids, respectively) all showed similar cytotoxicity to cells based on the CellTiter-Blue assay with significant toxicity at only 100 μg/mL Ag NP. $AgNO_3$ treatment caused comparable toxicity at doses similar to the free ions available in Ag NP solutions, suggesting that the observed toxicity is mostly due to free Ag^+. The undigested Ag NP and digested Ag NP with added food components were internalized by cells to a similar extent after 24-hour exposure to 20 μg/mL while significantly less of the digested Ag NP without the food components were internalized, likely due to the protection by the food components from Ag NP aggregation. Thus, it is important to not just investigate the effects of	135

			digestion on Ag NP toxicity, but also the presence of food components.	
< 20 nm (NM-300K) Ag NP stabilized with polyoxyethylene-glycerol trioleate and Tween 20	15-90 μg/mL, 3 hours	Caco-2 cells and the Caco-2/Raji B coculture M cell model	Metabolic activity of Caco-2 cells decreased in response to 3-hour treatment of cells with 30 μg/mL Ag NP (EC_{50} of 40 μg/mL). Ag^{+} at a concentration 10% of the total silver amount was cytotoxic to Caco-2 cells, but was less toxic than the Ag NP. Treatment of cells with the phenolic compounds (food matrix components) quercetin, kaempferol, and resveratrol in addition to Ag NP altered Ag NP toxicity. Quercetin (10 and 50 μM) and Ag NP restored cell metabolic activity to untreated control values, kaempferol was protective against Ag NP-induced decrease in metabolic activity at low doses although ineffective at higher doses, and resveratrol had no protective effect on Ag NP-induced toxicity. The protective effect of quercetin and kaempferol was also observed for the Ag NP-induced increase in ROS production (45 μg/mL), decrease in TEER, increase in Lucifer Yellow transport through mono- and co-cultures (30 μg/mL), and disruption of occludin and ZO-1 distribution. Benefits of this protective effect of phenolic compounds and the precise mechanism must be studied further.	136
PVP-capped 20 nm Ag NP (NM-300)	1.25-625 μg/cm^2, 24 hours	Differentiated THP-1 macrophages and MUTZ-3 dendritic cells were embedded in collagen on transwell inserts and Caco-2 cells were seeded on top	Ag NP treatment caused cytotoxicity in co-cultures at a dose of 156.25 μg/cm^2, but toxicity was observed in Caco-2 monocultures at 78.125 μg/cm^2. Induction of inflammation by treatment of the co-culture with IL-1β prior to Ag NP treatment slightly (but not significantly) increased toxicity at 312.5 μg/cm^2 Ag NP. Epithelial barrier integrity was disrupted after 24-hour 156.25 μg/cm^2 Ag NP treatment. The Caco-2 monoculture was the most sensitive to changes in TEER after Ag NP treatment, followed by the inflamed co-culture and finally the non-inflamed co-culture. There was a slight increase in IL-8 release in Caco-2 monocultures at 312.5 μg/cm^2, but increases in IL-8 release were observed in co-cultures at 39.065 μg/cm^2 Ag NP, with greater IL-8 release in the non-inflamed co-cultures. Thus, this co-culture system provides a model of the inflamed intestine for further NP toxicity studies.	54

Table 8S: Summary of *in vivo* chronic oral Ag nanoparticle exposure studies since 2012.

Ag NP characteristics	Dose and duration of exposure	Animal model	Major findings	Ref.
Rat models				
8-22 nm Ag NP	Oral administration of 100, 1000, and 5000 mg/kg/day Ag NP for 7 or 14 days. Additionally, 5000 mg/kg Ag NP was administered daily for 21 days.	Male Wistar rats	Oral administration of Ag NP to rats induced significantly decreased phosphate levels after 21-day treatment with 5000 mg/kg Ag NP. Serum creatinine levels increased significantly in a dose-independent manner in almost all treatment groups. Serum urea levels were significantly increased in the 21-day 5000 mg/kg Ag NP group only. Histopathologies including diffused inflammation, rapid differentiation, cellular degeneration, and necrosis were observed in the liver and kidney at all doses and treatment times. The elevated serum urea and creatinine levels, along with the observed histopathological damage indicate compromised kidney function, cellular morphology, and integrity.	137
20 nm citrate-stabilized Ag NP	Oral administration of 10 mg/kg/day Ag NP for 30 days. Rats were administered a single intraperitoneal dose of streptozotocin to induce diabetes prior to Ag NP treatment.	Six-month-old male Sprague-Dawley rats	Blood glucose levels were decreased by 68.2% after treatment with 10 mg/kg Ag NP, and serum insulin was increased by 3%. There was an increase measured in expression of insulin receptors and GLUT-2 as well as glucokinase activity (25.8%) in the liver of Ag NP-treated rats compared to untreated diabetic rats. Thus, Ag NP can act as an antidiabetic agent.	87
20 nm Ag NP in colloidal state in a protein protective	Oral administration of 820 mg/kg/day Ag NP for 81 days.	Weanling male Sprague-Dawley rats	Food intake was ~18% lower and body weight gain was 20% lower (final body weight was therefore also lower) in Ag NP-treated rats although there were no significant changes in organ weight. Plasma HDL cholesterol levels were unchanged, but	138

colloid as support, suspension contains 70-80% Ag			total cholesterol and LDL cholesterol were significantly increased after Ag NP treatment. The serum ratio of paraoxonase activity to HDL was reduced by ~15%, indicating oxidative stress. There was also an increase in plasma ALT activity. Superoxide production was increased by 30% in the liver and 41% in the heart. IL-6 increased by 12% and TNF-α increased by 9% in the liver compared to untreated controls. This led to the conclusion that the Ag NP are causing oxidative stress and inflammation in the liver and heart.	
14 nm polyvinyl pyrrolidone-coated Ag NP	Oral administration of 2.25 mg/kg, 4.5 mg/kg, and 9 mg/kg Ag NP daily for 28 days. A group of rats was also administered Ag acetate equimolar to 9 mg/kg Ag NP (14 mg/kg silver acetate).	Four-week-old Wistar Hannover Galas rats	Metabolomics analysis of urine revealed no difference between male rats treated with 9 mg/kg Ag NP/day and the untreated controls, but there were differences in Ag NP-treated females including a dose-dependent increase in uric acid and its degradation product allantoin. Only allantoin but not uric acid was increased after Ag acetate treatment. This suggests that Ag affects purine metabolism.	139
14 nm PVP-stabilized Ag NP	Oral administration of 4.5 mg/kg Ag NP to 6-week-old male rats once or twice daily (4.5 or 9 mg/kg/day) for 14 days to determine dose. In the 28-day study, 4-week-old male and female rats were orally administered 2.25, 4.5, or 9 mg/kg/day Ag NP. Ag acetate was administered to another group at 14 mg/kg/day, equivalent to 9 mg/kg	Wistar Hannover Galas rats	Oral administration of up to 9 mg/kg for 14 days did not cause changes in appearance, food intake, body weight, or pathology. A trend of lower body weight gain was observed over the 28-day study which was only significant for Ag acetate-treated rats. Ag acetate treatment increased plasma ALP and decreased plasma urea concentrations whereas Ag NP treatment did not. Ag acetate treatment also resulted in lower absolute thymus and liver weights, and relative thymus weights. The Ag NP-treated females showed decreased absolute thymus weights and males had lower absolute spleen and testes weights. The amounts of Firmicutes or Bacteroides in the cecum were unchanged and no pathological changes were observed after Ag NP or Ag acetate treatment. From this, they conclude that their results indicate a NOAEL level for 14 nm PVP-stabilized Ag NP of 9 mg/kg/day, the highest dose, while Ag acetate was	140

	Ag NP/day.		slightly toxic at a comparable dose.	
14 nm PVP-stabilized Ag NP	Oral administration of 2.25, 4.5, or 9 mg/kg/day Ag NP for 28 days. Ag acetate was administered at 14 mg/kg/day, equivalent to 9 mg Ag NP/kg/day. A 14-day administration of 2.25 or 4.5 mg/kg Ag NP was used to determine dose.	Female Wistar rats	Dopamine concentrations were increased in the brain following 28-day administration of Ag NP or Ag acetate although 14-day administration of Ag NP decreased the brain dopamine concentration. Noradrenaline was increased in brain following 28-day Ag acetate administration but not Ag NP administration. In contrast, the 5-HT concentration was increased following Ag NP but not Ag acetate administration. Based on this and an observed induction of apoptosis by Ag NP and Ag acetate *in vitro* in PC12 neuronal-like cells, they conclude that Ag NP are neurotoxic, potentially through release of Ag ions.	141
15 nm colloidal Ag NP in a protein matrix (~30% matrix and 70% Ag)	Oral administration of 500 mg/kg/day Ag NP for 30 or 45 days or gavage of Ag NP for 30 days followed by gavage of water only for 30 days. Another group was administered 820 mg/kg/day Ag NP for 81 days.	Weanling male Sprague-Dawley rats	A significant accumulation of Ag NP in the liver and kidney was observed after 45 and 81-day administration, with liver being the principal target. Ag NP localized to the cortex of the kidney with a large amount of Ag found in the renal artery while Ag NP were homogeneously distributed in the liver. Large (g/kg) amounts of Ag were excreted in feces which were largely in nanoparticulate form while low (μg/L) amounts of Ag were excreted in urine. A 30-day recovery period (after 30 days of Ag NP administration) allowed for the majority of the Ag to be excreted from rats. Ag was found to form complexes with various biomolecules in both liver and kidney including metallothionein and likely other cysteine-rich proteins.	142
10 and 25 nm citrate-stabilized Ag NP	Oral administration of 100 or 500 mg/kg/day Ag NP for 4 weeks. Rats were then sacrificed immediately or allowed to recover for 1, 2, or 4 months.	Five-week-old male and female Sprague-Dawley rats	No significant body weight differences or signs of adverse effects were observed during Ag NP treatment. Some increases in hepatotoxicity-relevant markers including cholesterol, ALP, and AST levels in serum were observed after Ag NP administration, but these levels returned to normal after a 2-month recovery period. Blood Ag concentrations decreased over the first month following the end of Ag NP treatment to levels which were maintained for the remainder of the 4-month recovery period with a half-life of 98.94 days (males) or 78.14	143

			days (females) for 10 nm Ag NP and 133.37 (males) or 140.12 days (females) for the 25 nm Ag NP. The Ag concentration gradually decreased in tissues including liver, kidney, and spleen over the 4-month recovery period. Ag seemed to persist longer in (or take longer to be cleared from) testes/ovaries and brain tissue, and this biopersistence needs to be a component of Ag NP risk assessment.	
70 nm Ag NP	Oral administration of 25, 50, 100, and 200 mg/kg Ag NP every 12 hours for 48 days (one spermatogenesis period).	Male Wistar rats at 45-50 days old.	Although not significant for all experimental groups, there were some increases observed in the percentages of live sperm with and without the acrosome reaction in Ag NP-treated rats, indicating that Ag NP can affect the acrosome reaction. Trends towards increased numbers of nonviable cells suggest that Ag NP may also affect sperm viability. Spermatogonia percentage was reduced in all treated groups, but only significant at 200 mg/kg. Significant reductions were observed in numbers of primary spermatocytes, spermatids, and spermatozoa, but there were no differences in Sertoli cell numbers. Only minor changes were observed in diameter of seminiferous tubules, but separation of primary spermatocytes and spermatogonia cells from tubule walls was observed at 100 mg/kg and 200 mg/kg and the release of sperm precursor cells to the mid-duct of the seminiferous tubules was observed at 200 mg/kg.	144
70 nm Ag NP	Oral administration of 0.25, 0.5, 1 and 2 mg/kg/day Ag NP for 30 days.	Eight-week-old male Wistar rats	Rats administered 2 mg/kg Ag NP displayed hair color changes (to a tan color). Spleen color changed and the spleen showed atrophy and damage at the 2 mg/kg dose. The red pulp in the spleen was observed to decrease and the white pulp to increase after Ag NP treatment. Necrosis of glomerular cells, Bowman's capsule, and proximal tubules was observed after treatment of rats with 1 or 2 mg/kg Ag NP, and proteinic sediment was observed in renal tubules. Inflammation of the parenchymal cells was observed in liver along with nuclear duplication and enlargement of intercellular space in cells of the hepatic lobule. Apoptosis was also observed around the	145

			central vein. Further studies are needed to fully understand the biological effects of Ag NP exposure.	
10 ± 4 nm colloidal citrate-stabilized Ag NP	Oral administration of 0.2 mg/kg/day Ag NP for 14 days. Another group was administered Ag citrate at the same dose (0.2 mg Ag/kg).	Male Wistar rats	ICP-MS studies showed that 0.2 mg/kg Ag NP and Ag^+ were absorbed from the gastrointestinal tract into the blood within hours after administration. Ag NP localized between lamellae of myelin sheaths and inside lysosomes of neurons and endothelial cells of microvessels. Pathological changes were observed in the hippocampus and forebrain cortex. Ag NP induced more severe changes in synapses, and free synaptic vesicles and myelin-like bodies were observed which were not present after Ag^+ treatment. Both Ag NP and Ag^+ induced swollen synapses in the neuropil and enhanced the density of synaptic vesicles within synapses. Ag treatment decreased the levels of synaptophysin, postsynaptic protein PSD-95, and synapsin I immunoreactivity. Thus, these data suggest Ag may be capable of causing synaptic degeneration.	146
17.7 ± 3.3 nm uncoated Ag NP (NM-300K) and 12.1 ± 8.0 nm PVP-coated Ag NP	Oral administration of 90 mg/kg/day Ag NP or 9 mg/kg/day $AgNO_3$ for 28 days. Rats were euthanized at 29, 36, or 84 days.	Eight-week-old male Sprague-Dawley rats	Blood Ag content was similar in groups treated with both sizes of Ag NP (90 mg/kg), but much higher in the $AgNO_3$ group (9 mg/kg) when normalized to Ag exposure dose, indicating a higher Ag uptake and suggesting that most of the absorbed Ag from the Ag NP suspensions is Ag^+. The Ag detected in feces of treated groups was estimated to be > 99% of ingested Ag. Ag levels were highest in the GI tract tissues followed by liver and spleen, and then testis, kidney, brain, and lungs. Ag washed out of most tissues to < 50% of the end-of-treatment levels after 1 week (day 36) and was almost completely cleared by day 84. However, Ag cleared much more slowly from brain, where levels were still > 90% at day 84, and in testis, where levels were > 70% at day 84 for $AgNO_3$ and uncoated Ag NP groups and > 30% for the PVP-coated Ag NP group. Spleen, liver, lungs, and GI contents were all found to contain Ag NP (rather than Ag in soluble form) in rats treated with Ag NP as well as $AgNO_3$, suggesting that Ag NP can form *in vivo*. Thus,	147

			exposure to Ag NP may be very similar to Ag^+, and the long retention of Ag in brain and testis needs to be considered in a risk assessment of Ag NP.	
7.9 ± 0.95 nm citrate-coated Ag NP	Oral administration of 250 mg/kg/day Ag NP to females for 14 days before mating, during the mating and gestation period, and for 4 days after parturition, and to males for 14 days before mating and during the mating period.	Sprague-Dawley rats	Ag NP were observed in liver, kidney, lung, and brain of pups from 250 mg/kg-treated dams, and kidney seemed to be the main target. Ag accumulation in kidney of treated pups was 12.3-fold greater than control, that in liver was 7.9-fold greater, that in lung was 5.9-fold greater, and accumulation in brain was 5.4-fold greater. This suggests passage of Ag NP from pregnant dams to offspring through the placenta.	148
10, 75, and 110 nm citrate-stabilized Ag NP	Oral administration of 9, 18, and 36 mg/kg Ag NP twice daily for 13 weeks. Some rats were also administered 100, 200, or 400 mg/kg Ag acetate/day.	Seven-week-old Sprague-Dawley rats	Greater antimicrobial activity of Ag NP on the microbiota was observed with decreasing nanoparticle size. While the 100 mg/kg dose of Ag acetate caused comparable effects to the low dose of 10 nm Ag NP, the 400 mg/kg Ag acetate doses caused the majority of rats to become moribund and the 200 mg/kg Ag acetate dose caused severe gastroenteritis. Treatment of rats with 10 nm Ag NP altered the proportion of *Firmicutes* (Gram-positive) to *Bacteroidetes* (Gram-negative) populations by decreasing the *Firmicutes* population, and also decreased the *Lactobacillus* population. Ag NP treatment caused a decrease in MUC3 expression in the ileum, a downregulation of TLR2, TLR4, and NOD2, and a decrease in expression of T cell regulatory genes (FOXP3, GPR43, IL-10, and TGF-β). The greatest changes in gene expression were observed at the lowest Ag NP dose (9 mg/kg) with the 10 nm Ag NP, and some differences were observed between males and females. These Ag NP-induced changes to the microbiota need to be investigated further to determine their potential health effects.	149
11 nm citrate-	Oral administration of	Five-week-old	In the 14-day study, the 1030.5 and 2061 mg/kg Ag NP doses	21

capped Ag NP	515.3, 1030.5, and 2061 mg/kg/day Ag NP for 14 days to determine doses. For the 13-week study, Ag NP were administered at 257.6, 515.3, and 1030.5 mg/kg/day.	female Sprague-Dawley rats	significantly increased ALP levels in males and all doses increased ALP levels in females, but no other parameters showed dose-related changes. In the 13-week study, ALP levels in both males and females increased at the highest Ag NP dose. The calcium levels in females were higher after Ag NP treatment at all doses. Lymphocyte infiltration was observed by histopathological analysis in liver in more of the high-dose Ag NP-treated males and females than in controls, and an increase in incidence of lymphocyte infiltration in kidneys was also observed in Ag NP-treated rats. These changes indicate potential liver and kidney toxicity. Ag NP treatment significantly increased Ag concentrations in blood as well as in all organs tested (liver, kidney, spleen, lung, and brain), but there was a particularly strong dose-response accumulation in spleen, lung, and brain. Ag concentration was greatly increased in the feces after Ag NP treatment, indicating that the majority of Ag was excreted.	
3-10 nm Ag NP	Oral administration of 1 or 10 mg/kg/day Ag NP for 14 days.	Seven-week-old female Sprague-Dawley rats	Rats orally administered 1 mg/kg Ag NP displayed neuron shrinkage, cytoplasmic or foot swelling of astrocytes, and extra-vascular lymphocytes. Gene expression of cadherin-1 and claudin-1 were slightly increased after Ag NP exposure, and IL-4 levels increased in serum. Thus, Ag NP may induce neurotoxicity through inflammation.	150
60 nm Ag NP	Oral administration of 15 or 30 μg/kg/day Ag NP from postnatal days 23-58. Rats were sacrificed at postnatal day 102.	Newly weaned (postnatal day 21) male Wistar rats	Prepubertal exposure to low doses of Ag NP (15 μg/kg) caused damage to the sperm plasma membrane and acrosome integrity in male rats and reduced mitochondrial activity. Thus, prepubertal exposure to Ag NP may alter the sperm and reproductive parameters in adulthood, and spermatic parameters must be evaluated to establish safety limits on Ag NP use.	151
5-20 nm citrate-stabilized Ag NP	Oral administration of 20 μg/kg/day Ag NP for 90 days.	Ten-twelve-week-old male Wistar rats	Oral administration of 20 μg/kg Ag NP for 90 days caused damage to testicular structure which progressed over time. Severely impaired and apoptotic germ cells were observed in	152

			the testis, and further study should be done on lower doses of Ag NP.	
< 100 nm Ag NP	Oral administration of 30 mg/kg/day Ag NP for 21 days during pregnancy (starting at embryonic day 0). Some rats were administered garlic juice in addition to Ag NP.	Female Wistar rats	Oral administration of 30 mg/kg Ag NP during pregnancy induced apoptotic cells in hippocampus of rat offspring. The co-administration of garlic juice was preventive and led to reduced numbers of apoptotic cells in the hippocampus of offspring.	153
14 and 36 nm gallic acid-capped Ag NP	Oral exposure to a solution of 535 µg/mL Ag NP *ad libitum* for 55 days and the Ag NP solution ingested was recorded for each treatment group.	24-day-old female Wistar rats	Oral administration of Ag NP to rats caused some changes in clinical chemistry and hematological parameters including blood urea nitrogen, total proteins, and mean corpuscular hemoglobin, but most were still within normal concentrations. Ag concentrations were highest in the small intestine, followed by kidney, liver, and brain. This study suggests that effects of Ag NP administration were not significantly dependent on NP parameters such as size.	154
7.9 nm citrate-capped Ag NP	Oral administration of 62.5, 125, and 250 mg/kg/day Ag NP for 42 days (males; 14 days before mating, 14 days during the mating period, and 14 days post-mating) or up to 52 days (females; 14 days before mating, during the mating and gestation period, and during 4 days of lactation).	Eight-week-old Sprague-Dawley rats	The only symptom observed following Ag NP treatment was alopecia in several rats, but this was not dose-dependent. No changes were found in mating, fertility, or pregnancy rate, gestation period, number of corpora lutea and implantation, delivery rate, number of live and dead pups, body weight of pups, or other pregnancy-related parameters examined.	155
7.5 nm Ag NP in 0.5%	Oral administration of 100, 300, or 1000	11-week-old Sprague-	The repeated oral administration of Ag NP during pregnancy caused oxidative stress in hepatic tissues at doses ≥ 100	156

carboxymethyl-cellulose	mg/kg/day Ag NP to pregnant rats from gestational days 6-19.	Dawley rats	mg/kg/day based on decreased levels of catalase and glutathione reductase in dams, but did not cause developmental toxicity up to 1000 mg/kg/day. Therefore, the NOAEL of Ag NP was < 100 mg/kg/day for dams and 1000 mg/kg/day for embryo-fetal development.	
< 100 nm Ag NP	Oral administration of 50, 100, 250, 500, and 1000 mg/kg/day Ag NP for 2 weeks.	Seven-week-old male Sprague-Dawley rats	Oral administration of Ag NP to rats had no effect on hepatic cytochrome P450 activities. However, it was shown that Ag NP could inhibit CYP2C and CYP2D *in vitro*, and thus the potential for Ag NP to inhibit cytochrome P450 enzyme activity should be further investigated when determining NP toxicity.	157
20, 50, and 100 nm spherical and 100 nm rod-shaped Ag NP	Oral administration of 35 mg/kg/day Ag NP for 23 days. Some rats were co-administered *W. somnifera* (35 mg/kg) with Ag NP.	Eight-nine-week-old Wistar albino rats	Administration of 35 mg/kg Ag NP induced liver and kidney damage, as evidenced by changes in serum biomarkers and histopathological changes. Spherical Ag NP displayed toxicity inversely proportional to NP size due to greater penetration of smaller NP. However, 100 nm rod-shaped Ag NP induced negligible toxicity in comparison to 100 nm spherical Ag NP. Treatment of rats with extracts of *W. somnifera* significantly reduced the 35 mg/kg Ag NP-induced toxicity, and thus *W. somnifera* extracts show potential for use as an antioxidant and anti-inflammatory agent against Ag NP.	158
Mouse models				
40 nm Ag NP	Oral administration of ~0.037 and 0.092 mg/kg/day Ag NP for 2, 7, and 14 days (50 μL of 20 or 50 ppm solutions of Ag NP).	Eleven-week-old BALB/c mice	Although red blood cells, hemoglobin, and hematocrit did not change after Ag NP treatment, treated mice did display higher white blood cell counts and lower monocyte numbers. AST levels increased after Ag NP treatment while ALT levels decreased at the higher dose but increased at the lower dose. Damage to liver tissue including necrosis, hepatocytic inflammation, and aggregation of lymphocytes was observed after Ag NP treatment. Thus, Ag NP were found to induce liver toxicity.	159
10-20 nm colloidal Ag NP	Administration of 0.25, 2.5, and 25 ppm in	Ten-twelve-week-old male	Ag NP treatment resulted in decreased monocyte percentages, total monocyte counts, and platelet levels. Mice given 0.25 and	160

	drinking water for 28 days.	NMRI mice	2.5 ppm doses of Ag NP had an increased percentage of $CD4^+/CD8^+$ double positive T cells and a decrease in the percentage of $CD3^-CD49b^+$ NK and $CD3^+CD49b^+$ NKT cells. There was an increase in the $CD4^+/CD8^+$ ratio in mice administered 25 ppm Ag NP. The lowest dose slowed the proliferation of B and T cells. The 2.5 ppm dose group had a decreased percentage of granulocytes engulfing bacteria and the 25 ppm group had a higher percentage of phagocytizing monocytes. The phagocytic activity of granulocytes increased after Ag NP treatment at all doses and there was an increase in the percentage of granulocytes and monocytes undergoing respiratory burst following bacterial stimulation. This suggests an importance of phagocytes in the immune response to nanoparticles.	
10-20 nm colloidal Ag NP	Administration of 0.25, 2.5, and 25 ppm Ag NP via drinking water for 28 days.	Ten-twelve-week-old male NMRI mice	The 0.25 ppm dose inhibited T cell proliferation and had a similar but not significant effect on the B cell response. The 2.5 and 25 ppm doses significantly stimulated B cell proliferation. In mice administered 25 ppm Ag NP, splenocytes secreted significantly less IL-6 and IFN-γ. The splenocytes from mice administered 0.25 and 2.5 ppm Ag NP secreted higher amounts of IL-1β, IL-2, TNF-α, IL-6, IL-10, and IFN-γ, although these increases were not always significant. LPS stimulation resulted in increased secretion of IL-1β in splenocytes from mice treated with 0.25 or 2.5 ppm Ag NP, and 2.5 ppm treatment also increased splenocyte secretion of TNF-α. Levels of IL-10 and IFN-γ were decreased in cells from mice treated with 0.25 or 2.5 ppm Ag NP. These effects of Ag NP on splenocytes suggest that lower doses could be more harmful than high doses and effects may be hard to predict.	161
10.15 nm Ag NP (size range of 3-20 nm)	Oral administration of 5, 10, 15, or 20 mg/kg/day Ag NP for 21 days.	Six-ten-week-old male Swiss albino mice	No differences were observed in food consumption, but a significant decrease in body weight was observed after Ag NP treatment for 2-3 weeks which was maximal at 10 mg/kg, and this dose was then used for further investigation. After Ag NP	162

			treatment, microvilli lining the intestine were lost or severely disrupted, there was an increase in inflammatory cells in the lamina propria which led to a widening of the lamina propria, and there was an increase in the number of mitotic figures in the intestinal glands. Thus, Ag NP exposure appears to destroy the small intestinal mucosa.	
20 nm Ag NP	Oral administration of 1 or 2 μM (0.1 or 0.2 mg/kg/2 mL/day) Ag NP for 14 days.	Male Swiss albino mice	A significant decrease in body weight was observed after exposure to both doses of Ag NP (~36% decrease). Ag NP treatment also increased ROS levels in blood and decreased GSH, GPx, and GST activities as well as IL-6 and NOS levels. DNA damage was detected based on an increase in 8-OHdG. In addition to blood, ROS also increased in all tissues tested (brain, liver, kidney, and spleen), the GSH/GSSG ratio decreased significantly, and GPx and GST enzyme activity was altered after Ag NP treatment. AST activity and urea levels were increased after Ag NP exposure, but ALT activity was unchanged. Ag NP induced metallothionein expression in liver, and especially kidney. Most of these effects showed a dose-dependent response. From this they conclude that Ag NP induced an oxidant-mediated response in mice.	163
Spherical < 100 nm PVP-coated Ag NP	Administration of 500 mg/kg/day Ag NP via drinking water or oral gavage for 5 days. Pregnant dams were administered Ag NP by oral gavage at 500 mg/kg/day for 5 days from 9.5 dpc to 13.5 dpc.	Three-four-month-old C57BL/6J p^{un}/p^{un} mice (recessive duplication of the *pink-eyed unstable (p^{un})* allele of the *pink-eyed dilution* gene which results in a light gray coat	The frequency of deletions in the retinal pigment epithelium during development was measured by administering Ag NP to pregnant dams. This treatment resulted in an increased rate of DNA deletions, suggesting that Ag NP ingestion can induce large-scale genome rearrangements in developing embryos. Ag NP exposure increased micronucleated erythrocytes and micronucleus frequency in bone marrow. There was an increase in double strand breaks in peripheral blood and bone marrow of Ag NP-treated wild type and *Myh*$^{-/-}$ mice, and 8-oxoG levels were increased in Ag NP-treated mice. Gene expression arrays detected the downregulation of 9 base excision repair genes and 6 homologous recombination genes. Several substrate-specific glycosylases and homologous	164

		and pink eyes) and $Myh^{-/-}$ mice in a C57BL/6J p^{un}/p^{un} background (Myh is a DNA glycosylase involved in base excision repair)	recombination or non-homologous end joining genes were found to be upregulated after Ag NP treatment. The DNA damage observed here suggests that Ag NP could lead to development of cancer over long-term exposure.	
Uncoated 25 and 100 nm Ag NP and < 25 nm PVP-coated Ag NP	Oral administration of 0.01, 0.1, and 1.0 mg/kg/day Ag NP for 7 days. Mice were also administered 20 mg OVA by oral gavage at day 7 following NP treatment to induce oral tolerance and immunized with 100 µg OVA by subcutaneous injection on day 14. A later experiment co-administered PVP-coated Ag NP and OVA by oral gavage for 7 days before immunization with OVA.	Six-eight-week-old BALB/c mice	Feeding OVA to mice prior to immunization suppresses the production of anti-OVA IgG, IgG1, and IgG2a antibodies due to induction of humoral immune tolerance. Administration of 0.1 and 1.0 mg/kg PVP-coated Ag NP prior to oral administration of OVA increased the anti-OVA antibody production after immunization. The 1.0 mg/kg dose of Ag NP increased antibody production to levels similar to those not tolerized with oral OVA administration, indicating that Ag NP blocked induction of oral tolerance. Accordingly, treatment with PVP-coated Ag NP increased the proliferative response of spleen cells, and increased their IFN-γ and TGF-β expression. Co-administration of PVP-coated Ag NP and OVA for 7 days by oral gavage was also found to block induction of oral tolerance. The effectiveness of Ag NP is likely due to Ag^+ since the PVP-coated Ag NP released ~10% of Ag in the form of Ag^+ whereas very few Ag^+ could be detected in the 25 or 100 nm uncoated Ag NP suspensions.	165
Human models				
5-10 nm and 25-40 nm (mean 32.8 nm) Ag_2O-coated	36 participants received 10 ppm of oral 5-10 nm Ag NP daily for 3, 7, or 14 days (~100 µg/day)	Human subjects 20-76 years old were enrolled.	Based on ICP-MS analysis, 84.3% of the Ag solution administered to subjects was Ag^+. Heart rate decreased significantly by 2.3 beats per minute for all Ag NP-treated subjects. Blood urea nitrogen and ALT levels were decreased	166

Ag NP	and 24 participants received 32 ppm of oral 25-40 nm Ag NP daily for 14 days (~480 µg/day).		after 10 ppm Ag NP treatment but were still in the normal range. Decreases in red blood cell counts after treatment with 10 ppm Ag NP were also not clinically relevant. The average peak serum Ag concentration was 1.6 ± 0.4 µg/L after 10 ppm treatment and 6.8 ± 4.5 µg/L was detected with 32 ppm. There was no Ag detected in the urine. There was indication of increased ROS production or proinflammatory cytokine production in sputum. No morphological or structural changes were noted after cardiac and abdominal MRIs. Further study is needed to determine human toxicity thresholds.	
25-40 nm (mean 32.8 nm) Ag NP coated with Ag_2O	Twelve participants received 32 ppm of oral Ag NP daily for 14 days (~480 µg/day)	Male subjects of age 18-50 were enrolled.	No clinically important changes were observed in weight, body mass index, blood pressure, heart rate, metabolism, blood cell counts, hematologic, or urinary analyses. A mean serum Ag concentration of 6.8 ± 4.5 µg/L was detected in all subjects and no silver was detected in the urine. There was no inhibition of cytochrome P450 enzyme activity after Ag NP administration although there was a non-significant decrease in the parent-to-metabolite ratio of dextromethorphan. Thus, Ag NP exposure does not appear to induce oxidative stress in liver tissue.	167
32.8 (range 25-40) nm Ag NP coated with Ag_2O	Participants received 15 mL of the 32 ppm Ag NP solution daily (480 µg/day) for 14 days.	Human subjects 20-56 years old	Ag NP ingestion induced a significant decrease (16.7%) in platelet aggregation in platelets from treated study participants in response to a collagen agonist. No spontaneous platelet aggregation was observed without the use of an agonist and this does not suggest increased platelet activation with Ag NP ingestion.	168
Other models				
13-19 nm SDS-capped Ag NP	Ag NP were administered mixed into tilapia feed at 0.8 mg/L and 0.4 mg/L. Fish were fed twice daily at 5% body weight of fish.	Tilapia early fingerlings	Thinned intestinal wall, partially blocked lumen, and thick mucosa were observed after 0.8 mg/L Ag NP administration while mildly blocked mucosal layer and a thin wall were observed after 0.4 mg/L administration. Ag NP dose-dependently increased catalase expression in intestinal epithelial cells. A dose-dependent decrease in microfloral count was detected after Ag NP treatment. There was a dose-	169

			dependent accumulation of Ag in the intestine. A slight initial increase in pH of the water was observed in Ag NP-treated groups, but no other water quality parameters were affected. Thus, toxicity was found to begin at 0.8 mg/L in tilapia and this study provides the basis for forming toxicological guidelines in aquatic ecosystems.	
70 nm Ag NP	Administration of Ag NP by supplementing food (*E. coli*) with 2.5 mg/L Ag NP suspended in water for 48 hours.	*Caenorhabditis elegans* wild-type N2	Ag NP treatment at 2.5 mg/L for 48 hours did not cause accumulation of insoluble ubiquitinated proteins or protein aggregation, and thus Ag NP do not seem to affect protein homeostasis.	33
35 nm Ag NP and 0.6-1.6 μm Ag microparticles	*Daphnia magna* exposure in water containing 0.01, 0.1, 1 and 10 mg/L Ag NP for 96 hours and 0.001, 0.005, 0.01, and 0.5 mg/L Ag NP for 21 days. Carp were exposed to 0.01 and 0.1 mg/L Ag NP for 21 days.	< 24-hour *Daphnia magna* neonates and carp (*Cyprius carpio*)	Ag NP caused concentration-dependent mortality in *Daphnia magna* neonates in the 96-hour study beginning at 0.1 mg/L (57% mortality). In the 21-day exposure study, the lowest dose of Ag NP, 0.001 mg/L, showed the highest mortality (30% after 21 days). After 21-day exposure of Ag NP to carp, significant Ag uptake was detected in liver, intestine, and gallbladder although there were significant differences between individual fish. A trend of increased Ag concentration was also observed in gills, but Ag NP ingestion seemed to be a more significant means of NP uptake than respiration. Ag NP were found to be more toxic than Ag microparticles.	134
58.6 nm Ag NP	Oral exposure through diet at 500 mg Ag NP/kg feed, and fish were fed twice daily at 3% body weight/day for 14 days. AgCl was also incorporated into feed at 500 mg Ag/kg as a control.	Wild type zebrafish	Zebrafish gut microbiota displayed minor changes in community richness and diversity in response to oral administration of 500 mg/kg feed Ag NP. Similar disruption was observed with Ag^{+}. Future studies should focus on dose-dependent effects, duration of disruption after exposure, and effects on host health.	170

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