The Effect of Size on Uptake of Orally Administered Latex Microparticles in the Small Intestine and Transport to Mesenteric Lymph Nodes

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Purpose. The present study examines the relationship between size and particle transit across the mucosal barrier of the gastrointestinal tract to other sites of the body. The extent of particle uptake with increasing size, the tissue distribution and cut-off points for 2–20μm particles is investigated.

Methods. An established fluorescent latex particle-young adult rat model is used and particle numbers in small intestine and mesenteric lymph nodes, 0.5 h post administration, counted by fluorescence microscopy in bulk tissue specimens and cryosections.

Results. Bulk tissue analysis provides evidence for the presence of particles of all sizes in the Peyer's patch regions, but only for 2 μm particles in the nodal tissues. Microscopy establishes uptake of both 2 and 6 μm particles in most intestinal and nodal tissue sites and compartments. By contrast, uptake of the larger particles is much reduced.

Conclusions. Although more of the smaller (2 μm) particles are taken up, particularly by epithelial tissues, the 6μm size appears more efficient in terms of volume translocated to lymph nodes. This could have implications in the therapeutic use of particles for drug and vaccine delivery and for radiation safety.

KEY WORDS: microparticles; particle size; intestine; mesenteric lymph node; fluorescence microscopy.

INTRODUCTION

Most reports now agree that, following experimental administration, particles in the micrometer range may be taken up by the gastrointestinal mucosa and moved onwards within the body tissues (1–4). There is still debate about the exact mechanisms involved and the influence on the process of the characteristics of the particles, including their physicochemical nature and their physical dimensions. There have been several reports on the relationship between size and particle uptake (5–9). Study of the importance of size, like all other aspects of particle uptake, is complicated by the variation in detailed protocols used and in the extent of particle uptake, both across individuals and down any individual gastrointestinal tract (10). There is, however, general agreement that the extent of uptake is indirectly proportional to particle size, with very few large particles crossing the mucosal barrier: despite this, it is not clear at what size the cut-off point for uptake occurs. There have also been few descriptions of the effects of size variation on particle distribution across different tissue compartments. The current paper uses established techniques (11) to comment on this and on the relationship between particle numbers and other physical parameters of the particles.

MATERIALS AND METHODS

Non-fasted male Sprague-Dawley rats, 7–8 weeks of age and weighing approximately 250g were maintained on standard laboratory food and tap water available *ad libitum*. Plain (non-ionic) monodisperse fluorescent (YG) polystyrene latex microspheres of different sizes (nominally 2, 6, 10 and 20μm) were obtained from Polysciences Inc (Warrington, PA, USA). Experimental groups of animals (n = 6 per group: 3 animals for bulk and 3 animals for microscopic analysis) were given single orally instilled doses of 0.25ml undiluted latex suspension, delivered by 1ml syringe to the pharyngeal region. The particle sizes and the estimated particle numbers administered to animals in each experimental group are shown in Table 1 with each animal receiving an estimated 6.25 mg of polymer. Control animals (n = 6 per group: 3 animals for bulk and 3 animals for microscopic analysis) received 0.25ml sterile double-distilled water. Animals were killed 0.5h post-particle administration by carbon dioxide asphyxiation. All treatments to animals were carried out by a licensed investigator in accordance with regulated procedures which adhere to the "Principles of Laboratory Animal Care" (NIH Publication #85-23) by using protocols in line with the Animal (Scientific Procedures) Act (1986).

Tissue samples were taken either unfixed for bulk maceration, or removed following whole animal perfusion-fixation with 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) at ambient temperature for microscopy. The mesenteric lymph nodes (MLN) were, in each case, removed first, prior to excision of the small intestine.

For maceration, the proximal third of the small intestine and MLNs were removed. All Peyer's patch regions (PPR), a term applied to the excised lymphoid epithelial domes and any residual adjacent villous tissues (11), were removed from the proximal intestine and pooled (n = 3–7). All PPRs and MLN tissues were weighed, digested in 15ml of 15% KOH at 60°C for 2–3 days, diluted to 25ml with 2% KOH and vortexed for 1 min prior to particle counting. Particle numbers in twenty 1μl aliquots were determined, the average number per μl aliquot established, and an extrapolated value for the number of particles per μg tissue made.

For microscopy, the proximal ninth of the small intestine and MLN tissues were analysed: intestinal tissue was dissected to give Peyer's patch-containing (PPC) full circumference slices of the intestine. The uptake of particles into different tissue sites of the PPC tissues and their transit into MLNs were assessed in propidium iodide-stained (0.001mg/ml in 0.1M sodium cacodylate buffer for 30 min) 14μm thick cryosections mounted, without dehydration, in Gelvatol (Cairn Chemical, Chesham, Bucks, UK). Particle distribution across individual sites was analysed by epifluorescence microscopy: the sections were coded prior to analysis. The variable quality of particle-laden cryosections made it difficult to use truly random sampling techniques: therefore, the first 10 good quality sections were

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ABBREVIATIONS: FAE: Follicle-associated epithelium; PPR: Peyer's patch region; PPC: Peyer's patch circumference; MLN: Mesenteric lymph node; KOH: Potassium hydroxide.

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Table 1. Particle Numbers in Bulk Specimens

Parameter	2 μm	6 μm	10 μm	20 μm
Dose of particles administered	1.88×10^9	6.02×10^7	1.3×10^7	1.21×10^6
Mean Particle counts in PPRs ($\times 10^3$)	13.7 ± 7.9	0.7 ± 0.6	0.07 ± 0.06	0.002 ± 0.002
Percentage of administered dose (per μg PPR tissue)	0.7	0.7	0.7	0.7
Mean Particle counts in MLNs ($\times 10^3$)	0.98 ± 0.47	0	0	0
Percentage of administered dose (per μg MLN tissue)	>0.001	0	0	0

Note: Data show (i) mean particle numbers (and standard errors) per μg tissue proximal Peyer's patch regions and pooled mesenteric lymph nodes (n = 3–5) and (ii) estimate of concentration of particles per μg tissue as a percentage of the administered dose.
[a] Significantly higher (p = 0.02) numbers of 2μm than 20μm particles in PPR tissues.

used from within a pool of 30, giving a total of 140 μm of tissue sampled for each animal. Optical sectioning using confocal scanning laser microscopy provided further evidence of particle localisation within the tissues.

Differences between data sets were assessed for statistical significance using a one-way analysis of variance and the Scheffe-F test of multiple comparisons between pairs of means using a minimum probability significance value of p = 0.05. Counts for both bulk and microscopical samples were spot checked by a different observer to assess the effects of inter-observer variation and fading of particle fluorescence. Latex volume and epithelial surface area covered were calculated from $4/3\pi r^3$ or πr^2 respectively, where n = number of particles and r = particle radius.

RESULTS

Bulk tissue analysis (Table 1) showed particles of all four sizes in PPR specimens: there were no 6, 10 or 20 μm particles in nodal tissues. Some artefactual contamination of control samples occurred in the 2μm particle group with counts of 0.008×10^3 recorded. For 2μm particles, the mean number of particles counted in twenty 1μl aliquots of macerated PPR tissue ranged from 17–172; for 6μm particles, 0.6–2; for 10μm particles, 0–1.5; and for 20μm particles, 0.1–0.6. Although this inter-animal variation in the experimental data restricted the extent of statistical significance, the trends suggested that more 2μm particles than any of the larger sizes were present. Data from both bulk and microscopical analyses did not appear to be affected by inter-observer variation or fading of particle fluorescence.

Microscopical analysis showed that for each experimental group administered a given particle size, the particles were localised in a range of tissue sites (Figure 1). Although many of the particles were found in luminal, intervillous and mucosal surface regions, they were also found, in varying numbers, in several tissue sites (Table 2). The presence of greater numbers of smaller particles in some tissue sites (Table 2) and compartments (Table 3) was observed: many sites were free of 10μm and almost all sites were free of 20μm particles. Despite inter-animal variation, statistical analysis of the data revealed that 2μm particles appeared in significantly greater numbers in enterocytes and the epithelial compartment: 6μm particle numbers were however, significantly higher in broken nodal tissue (Table 2).

When other physical parameters of the particles were considered (Table 3), it was found that the large number of 2μm particles taken up was counterbalanced by their comparatively small dimensions, with 6μm often being the optimum size by volume, particularly for translocation to lymph node. It was also found that for 10μm particles, there was a trend for a substantial volume of latex to be associated with goblet cells.

DISCUSSION

Much of the literature on microparticle uptake is based on particles with diameters in the 1–2μm range (1–3). The present study is based on a basic model of fluorescent polystyrene latex particles given to non-fasted young adult rats (11) where, for 2 μm particles, the maximum uptake over a 24 h period has been shown to occur within 0.5 h after administration, thereby justifying the use of this time-point in this investigation. Changes in transport properties and gastrointestinal motility produced by fasting (12) further rationalise the use of animals given food and water *ad libitum*. The data presented here confirm the earlier findings with both bulk tissue and microscopic analysis identifying 2 μm particles in Peyer's patch regions of the proximal small intestine and in lymph node 0.5h after administration: the morphological pattern of uptake in small intestinal compartments is found to be similar to that seen in the previous report (11).

The estimation of the relationship between uptake and volume ingested is not straightforward. The maceration data may overestimate uptake into tissues since the microscopical results show that, for small intestine, most particles are luminal or on the surface. In addition counts of a total of twenty 1μl aliquots taken from 25ml of tissue macerate may also contribute to the potential overestimate. The proportion of the administered dose per μg taken up probably lies between 0.1 and 1% for the intestine and is lower for the lymph node: although direct comparisons are difficult, these figures are similar to those previously quoted by Ebel (6), lower than those of Jani et al (13) but higher than those of Jenkins et al (8). The variation in percentage uptake may be related to the different protocols used. It is noteworthy in the present study that the percentage of the administered dose per μg of tissue is similar for all 4 particle sizes, despite the fact that a larger number of smaller particles were given to produce approximately equal bolus size.

The identification of size cut-off points beyond which uptake stops has been explored in a number of studies (5–8, 14), where there is evidence that particles of different diameters are taken up to a varying extent, giving an uptake gradient with particle size. In the current study, a broader view is provided by bulk tissue analysis, whereas the more time-consuming microscopical analysis is more sensitive. Despite inter-animal variation (10) reducing the number of significant data set com-

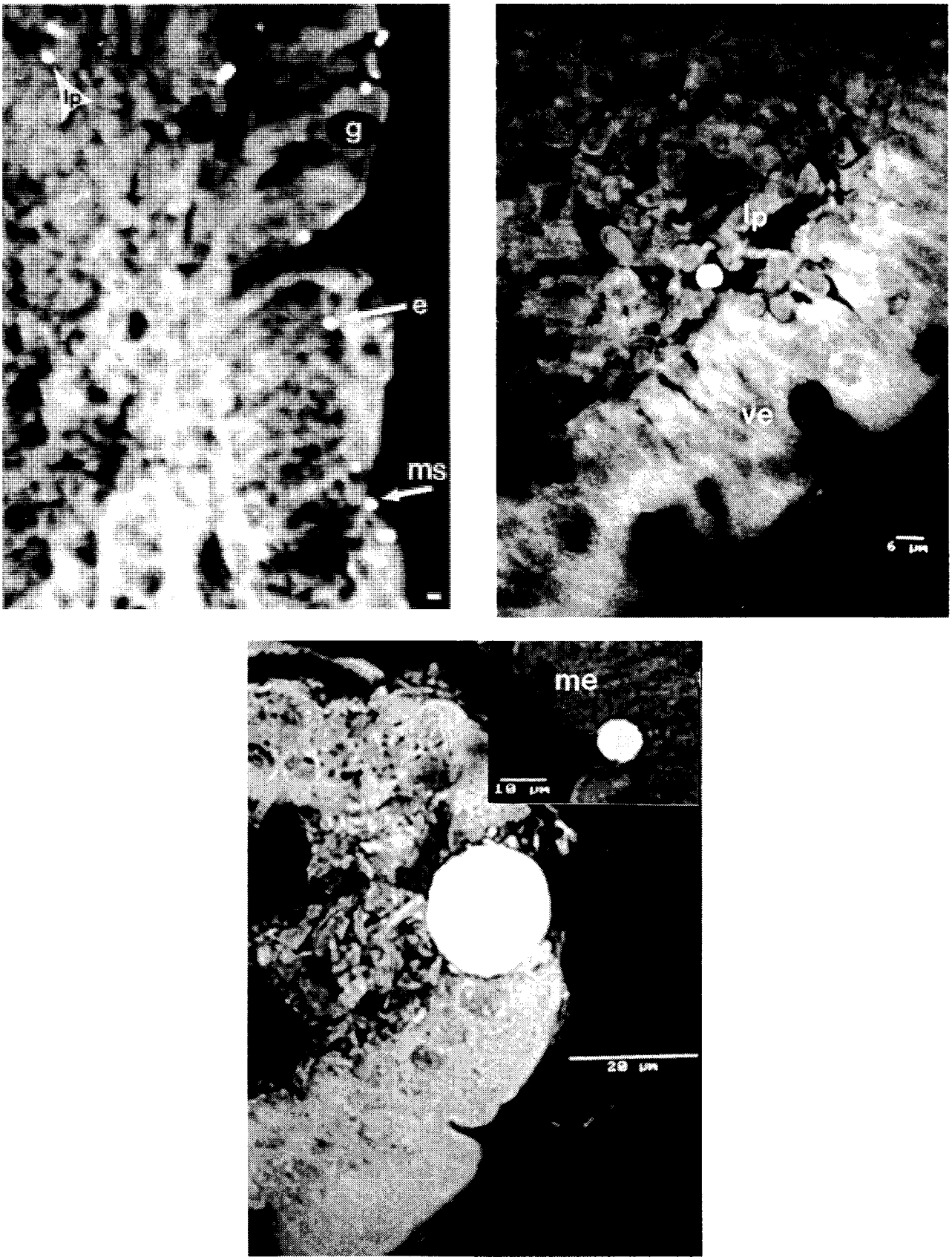

Fig. 1. (A–C) Confocal images of rat small intestine showing: [A] 2μm particles (arrows) at the mucosal surface of enterocytes (ms), within enterocytes (e) and within the villous lamina propria (lp), a goblet cell is also shown (g) (bar: 2μm); [B] a 6μm particle near the interface of the villous epithelium (ve) and lamina propria (lp); [C] a 20μm particle close to the villous tip and a 10μm particle (insert) associated with the mucosal epithelium (me).

Table 2. Particle Distribution in Individual Tissue Sites

TISSUE SITE	PARTICLE COUNT 2μm	6μm	10μm	20μm
Proximal Small Intestine				
Lumen[a]	86.7 (19–246)	21.4 (4–66)	2.7 (0–13)	0.2 (0–2)
Intervillous	98.6 (10–205)	2.6 (0–7)	1.2 (0–6)	0.7 (0–16)
Mucosal surface enterocyte	55.8 (18–121)	5.6 (0–14)	0.2 (0–1)	0.2 (0–4)
Mucosal surface goblet cell	1.4 (0–4)	0.1 (0–2)	0.04 (0–2)	0 —
Villous enterocyte[b]	33.4 (7–55)	1.6 (0–3)	0.1 (0–2)	0.01 —
Villous goblet cell	0.9 (0–5)	0.03 (0–1)	0.08 (0–2)	0 —
Villous lamina propria	10.5 (0–52)	0.8 (0–6)	0.08 (0–2)	0 —
Cryptal epithelium	1.3 (0–13)	0 —	0 —	0 —
Pericryptal stroma	0.8 (0–10)	0 —	0 —	0 —
FAE	0.2 (0–2)	0.2 (0–3)	0 —	0 —
PP Lymphoid tissue	0.4 (0–2)	0.3 (0–2)	0 —	0 —
Submucosa	0.2 (0–2)	0 —	0.01 0.01	0 0
Deep blood vessels	0 —	0.1 (0–1)	0 —	0 —
Muscularis externa	0.5 (0–2)	0.3 (0–2)	0 —	0 —
Serosa	0.6 (0–3)	0.1 (0–1)	0 —	0 —
Mean total	291.3	33.2	4.3	1.1
Mesenteric Lymph Node				
Capsule	2.7 (0–6)	0.5 (0–1)	0 —	0 —
Parenchyma	3.3 (0–7)	1.0 (0–2)	0 —	0 —
Blood/blood vessel	2.0 (0–4)	2.5 (1–2)	0 —	0 —
Near/broken[c]	2.0 (1–3)	5.5 (1–11)	0 —	0 —
Mean total	10	9.5	0	0

Note: Data show for each individual tissue site the mean number of particles counted per 14 μm section. Mean totals are total particle counts from all sections divided by the number of sections counted (n = 30).

[a] Significantly greater (p = 0.004) numbers of 2μm than 6, 10 or 20μm particles.

[b] Significantly greater (p = 0.004) numbers of 2μm than 6, 10 or 20μm particles.

[c] Data showed 6μm particle numbers significantly greater (p = 0.014) than 10 and 20μm.

Table 3. Particle Numbers and Volume in Tissue Sites and Compartments

	2μm	6μm	10μm	20μm
Mean particle number in tissue compartment per section				
Luminal and Surface[a]	242.5	29.8	4.1	1.1
Epithelial[b]	35.8	1.9	0.2	0.01
Non-epithelial	13.0	1.5	0.1	0
Estimated volume of latex in small intestine and mesenteric lymph node				
Volume (μm^3) of 1 particle	3.2	99.0	443.3	4916.2
Total latex volume in epithelial & non-epithelial tissue (μm^3)	156.1	333.3	116.8	49.3
Latex volume in total epithelium	114.6	188.1	78.3	49.3
Latex volume in enterocytes	106.9	158.4	42.9	49.3
Latex volume in goblet cells	3.1	3.3	35.5	0
Total non-epithelium	41.5	145.2	38.4	0
Lamina propria	33.5	75.9	34.0	0
Lymph node[c] (see Table 2)	32.0	940.5	0	0
Surface Area of small intestine				
Area covered by 1 particle (μm^2)	2.6	25.9	70.3	349.5
Total number on mucosal surface	56.3	5.7	0.2	0.1
Total Mucosal surface covered by particles (μm^2)	148.9	149.4	14.8	51.3
Mucosal surface Enterocytes (μm^2)	145.2	145.9	11.7	51.3
Mucosal surface Goblet cell (μm^2)	3.7	3.5	3.1	0

Note: Data show estimates of latex volume and mucosal surface area covered by latex.

[a] Significantly greater (p = 0.01) numbers of 2μm than 10 or 20μm in the luminal compartment.

[b] Significantly greater (p = 0.002) numbers of 2μm than 6, 10 or 20μm in the epithelial compartment.

[c] Significantly greater (p = 0.01) volume of 6μm than 2, 10 or 20μm in lymph node.

parisons, both techniques used here show similar cut-off or uptake gradients, although some details are not identical for the two sets of data: the current microscopy results show a cut-off for lymph node beyond 6μm, as opposed to the bulk data which detect no particles of this size. This dependence on technique may explain the different reports in the literature, with some describing particles around 3μm as immobilised within the Peyer's patches (13) and others describing them as found in the mesenteric lymph nodes (8). Comments below on cut-off or uptake gradient concentrate on the more detailed epifluorescence data sets for proximal small intestine and nodal tissues.

There is an uptake gradient in the epithelial compartment, with the larger particles appearing in significantly lower numbers than the 2μm particles, localising some of the previously reported size constraints to this compartment (6–8,13,15). A few individual sites display either an uptake gradient or a cut-off between 2 and 6μm. There is a gradient for enterocytes,

with significantly more 2μm particles: above this size cryptal compartments also show a cut-off. Comparison of 6μm particle uptake with that of larger particles shows that for the latter, there is a cut-off for intestinal lymphoid tissue, several deeper gut layers and lymph node. Beyond 10μm, there is a near total cut-off for epithelial tissues and a complete cut-off for non-epithelial tissues, including villous lamina propria, confirming reports of exclusion of particles larger than 10μm by the intestinal mucosa (5,7). The general pattern for cut-off points and significant gradients confirms the inverse relationship between particle size and numbers taken up and, as particle size increases, the number of sites involved decreases substantially. While there is clear evidence for microparticle uptake by the gastrointestinal tract the mechanism(s) involved and the size selectively in particle uptake is unclear. Although uptake of macromolecules, particulate antigens and other nanoparticulates have been ascribed to the activities of the specialized M-cells of the Peyer's patches (1,2) it is not at all certain, as yet, that this is the major route for microparticles and recent evidence points to a transport pathway across the villous enterocytes (11). Several modes of transmucosal uptake have been described (transcellular and paracellular transport, as well as via M-cells of the Peyer's patches) which may selectively govern intestinal uptake of particles of different size. Tables 2 and 3 show that there is virtually no uptake of the larger particles (10 and 20 μm) across the FAE although there is some villous epithelium uptake of 10 μm particles, perhaps suggesting that the FAE is more concerned with uptake of smaller particles, implying M-cell involvement, whereas larger particles may use the villous pathways, either transcellular or paracellular: further studies will be needed to address this issue in greater detail.

When particle dimensions are considered, the fact that each 6μm particle is so much larger, ensures that it is the most efficient size for introducing a given volume into tissues and its translocation on to the lymph nodes. This identification of 6μm as the possible optimum size for uptake has positive implications for therapeutic schedules, where particles are used to transport pharmacological agents into body tissues: it would appear that 6μm particles could deliver a high volume to target organs. The model also highlights more negative implications, where exposure to nuclear emissions of this size could lead to substantial uptake of particles with the possibility of enhanced radiation-induced damage. In conclusion, it is therefore clear that particle numbers and size alone do not give complete profiles of the likely effects of administration: further work is needed on the effects on uptake into different tissue compartments of variation of other particle characteristics, such as the composition and hydrophobicity of the material.

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