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Factors influencing intestinal microparticle uptake *in vivo*

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Abstract

The aim of this study is to compare microparticle uptake in animals of different ages, gender and species and at different time points. The 2 μm latex/*in vivo in situ* model uses the observation of animal responses or post-mortem changes and also particle identification by fluorescence microscopy in nine sequential intestinal segments and secondary sites. The wide size range of animals studied requires particle numbers in tissue compartments to be related to intestinal tissue section area through a circumference measurement. Area under the curve (AUC) data for particles in intestinal tissue are plotted against measurements of intestinal length, allowing comparisons to be made across different ages and species and between males and females. The percentage uptake of administered dose and particle numbers in macerated tissue are also reported. Some parameters, in particular species, do not appear to affect the extent of microparticle uptake, which ranges from 0.12 to 0.32% of the administered dose. Particle uptake does, however, vary with age, being significantly greater in young adult males (7 weeks) than in younger (3 weeks) and older (17 and 52 weeks) age groups. It is concluded that age is more important in determining the extent of uptake than gender or species.

Keywords: Microparticle uptake; Rat small intestine; Age; Species; Time

1. Introduction

Microparticles and nanoparticles cross the intestinal epithelial barrier into body fluids and other sites (Jenkins et al., 1994; Rubas et al., 1995; Simon et al., 1995; Jani et al., 1996; Seifert et al., 1996; Florence, 1997; Hussain et al., 2001), but the route, mechanism and extent to which this occurs are not yet entirely clear. The phenomenon is relevant to the optimisation of antigen and drug delivery (Eatock et al., 1999; Ravi Kumar, 2000; Florence and Hussain, 2001; Krauland and Bernkop-Schnurch, 2004; Lamprecht et al., 2004a,b). It is also relevant to the uptake of ingested pollutants (Bhattacharyya, 1983; Lang and Raunemaa, 1991; Harrison and Stather, 1996) and to the multi-organ dysfunction syndrome (MODS; Nieuwenhuijzen et al., 1996; Johnson and Mayers, 2001; Wiesner et al., 2002). Microparticle uptake is also important in the transmucosal passage of bacteria (Ohsugi et al., 1996), which is known to be affected by external radiation (Chun et al., 1997; Weichselbaum, 2005).

The wide range of reported particle uptake, from 0.01% (Ebel, 1990) to 3.6% (Jani et al., 1990), may be due to the non-standard nature of the protocols used (Delie, 1998) and more specifically to variations in species (Seifert et al., 1996), methods of particle administration (LeFevre et al., 1989), time points (Sanders and Ashworth, 1961; LeFevre et al., 1980; Jani et al., 1990; Sass et al., 1990; Jenkins et al., 1994; Hodges et al., 1995a; Limpanussorn et al., 1998) and assay methods (Neutra et al., 1987; Jani et al., 1989; LeFevre et al., 1989; Ebel, 1990; Limpanussorn et al., 1998). The current study focuses particularly on the accurate quantification of intestinal uptake, identified as 'crucial' by O'Hagan (1996). Some of the techniques developed in a previous study, reporting higher uptake in late pregnant and early lactating animals (Smyth et al., 2005), are used here to explore an appropriate time point after particle administration for the identification of further 'at risk' groups such as younger as opposed to older individuals. The study of uptake through intestinal walls from animals of different sizes could give some information, although without reference to other physiological variations, on whether it is possible to extrapolate to other larger species, such as humans. Methods are described for taking into account the range of intestinal diameters involved in the study of different age groups (Hollander

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and Tarnawski, 1985; Katz et al., 1987; LeFevre et al., 1989; Simon et al., 1994; Seifert et al., 1996) and species (Pappo and Ermak, 1989; Harrison and Fritsch, 1992; Howard et al., 1994; Ermak et al., 1995; Naylor and Harrison, 1995). The aim is therefore to use these methods in combination to give a clear picture of site specific particle uptake, percentage of uptake relative to administered dose and particle movement over time, in order to enhance further the comparisons made across gender, species and age.

2. Materials and methods

2.1. Experimental design

2.1.1. Experiments

Four particle uptake experiments explored the effects of time, gender, age and species (experiments 1–4; Table 1). All were carried out by licensed investigators, in accordance with regulated procedures under the Animals (Scientific Procedures) Act 1986. All animals were non-fasted, housed in a 12-h light:12-h dark photo-period and maintained on standard laboratory food and water *ad libitum*. The hypothesis that 30 min was an appropriate sampling point was tested by comparing uptake in one proximal and one distal segment from baseline, young adult (7 weeks) Sprague–Dawley rats at three time points after particle administration: these were 5, 30 and 90 min. The age study used male Sprague–Dawley rats 3, 7, 17 or 52 weeks of age, with gender comparisons carried out on 7-week-old male and female Sprague–Dawley rats. The species study used young male adult CFLP mice (aged 7 weeks), Sprague–Dawley rats (7–8 weeks) and Duncan–Hartley guinea pigs (11–12 weeks).

2.1.2. Particles and sampling

Plain (non-ionic) yellow-green (YG) fluorescent polystyrene latex microspheres (2.5% solid latex in distilled water) with YG dye excitation maxima of 458 nm and emission maxima of 540 nm (Polysciences, Warrington, PA, USA) were stored at 4 °C in 5 ml vials. Particle diameter was 2 μm, although this varied slightly between batches. Dosage for rats and guinea pigs was 1.42 to 1.95×10^9 particles in 0.25 ml and for mice, the smallest species, 6.84×10^8 in 0.1 ml. Mice and rats were gavaged, but administration to guinea pigs was through a tube to the pharynx, due to the unavailability of a suitable gavage needle. Animals had access to chow and water before and after particle administration. Data from animals receiving particles ($n \geq 5$) were compared with corresponding control groups ($n \geq 3$), exposed to the appropriate amount of sterile double-distilled water (Table 1).

Appropriate measures were taken to avoid cross-contamination from particle-fed to control-fed animals. To confirm the basic soundness of the methodology, observations were made of animal response during particle administration and of the visibility of green fluorescent particles in organs at post-mortem or during sample preparation. Specimens were coded to enable 'blind' counting of particles.

2.2. Experimental method

Animals for time experiments were asphyxiated with carbon dioxide at 5, 30 and 90 min after particle administration, before perfusion fixation with 3% buffered glutaraldehyde through the left ventricle. All other experimental groups (gender, age and species) were asphyxiated at the 30 min time point. Mesenteric lymph nodes and small intestine were removed and the length of the latter measured. It was then divided into nine equal segments, from which full circumference samples were selected, mostly from Peyer's patch-containing areas. The intestinal lumen was washed with 0.1 M sodium cacodylate buffer.

After luminal washing, intestinal samples were immersed in liquid nitrogen-cooled isopentane, cut on a cryomicrotome into sections 14 μm thick and mounted on APES-coated slides. Sections were then stained with propidium iodide, mounted and protected with coverslips. Mesenteric lymph nodes were prepared in the same way.

Particle numbers in small intestine and mesenteric lymph nodes were estimated by fluorescence microscopy in a minimum of 3, 14 μm sections per group. Intestinal particles were allocated to one of the following compartments: luminal (luminal and inter-villous); mucosal surface (surface villous enterocytes and surface goblet cells); villous regions (villous enterocytes, goblet cells and lamina propria); cryptal and deep regions (cryptal epithelium, pericryptal stroma, submucosa, blood vessels, muscularis externa and serosa); Peyer's patches (follicle-associated epithelium (FAE) and lymphoid tissue). For total uptake, numbers were added for all sites apart from those in luminal and mucosal surface compartments. Total uptake for the small intestinal length was estimated by multiplying the sum of this figure for all nine segments by intestinal length divided by 14 μm, the thickness of sectioned sample counted (Smyth et al., 2005): total uptake divided by the number of particles administered gave the percentage of administered dose taken up.

2.3. Particle content per mm^2 tissue as observed by microscopy (experiments 2–4)

Using a camera lucida, a representative section from each of the nine intestinal segments was traced for each animal, scanned into the computer and imported into the National Institute of Health Image Processing and Analysis programme (NIH Image 1.2) to give a figure for the total tissue area of the sections, which reflects the 'thickness' of the wall which takes into consideration the thickness of the tube and also the overall diameter.

2.4. Calculation

This measurement allowed the calculation of 'tissue loading' in particle numbers/mm^2. By plotting the tissue loading/mm^2 (Y-axis) calculated for each segment against the intestinal length in centimetres (X-axis) a measurable 'area underneath the curve' (AUC) was produced, allowing comparison of uptake efficiency across groups. This, in turn, provided an 'uptake profile' to be displayed graphically. This AUC information for uptake

Table 1
Details of experiments, animal groups and samples

Experiment number and variable studied	Gender	Species	Age in weeks	Total number of animals/group[a] (30 min after administration, unless otherwise stated)	Number of experiments/group[a] (30 min after administration, unless otherwise stated)	Samples assayed[b]
(1) Time microscopy	Female	Rat	8	7 P[*] (5 min) 7 P[*] 3 P (90 min) 2 C[*] (5 min) 1 C	3 P 3 P 1 P 2 C 1 C	SI, segments 2 and 8
(2a) Gender microscopy	Male, female	Rat	7	6 P 3 C	2 P 2 C	SI, segments 1–9 MLN
(2b) Gender maceration	Male, female	Rat	7	3 P 3 C	1 P 1 C	SI, proximal, middle, distal MLN
(3a) Age microscopy	Male	Rat	3, 17, 52 (+7 weeks, expt 2a)	6 P 3 C	2 P 2 C	SI, segments 1–9 MLN
(3b) Age maceration	Male	Rat	3, 17, 52 (+7 weeks, expt 2b)	3 P 3 C	1 P 1 C	SI, proximal, middle, distal MLN
(4a) Species microscopy	Male	Mouse, guinea pig (+rat, expt 2a)	7, 11 (young adult)	6 P 3 C	2 P 2 C	SI, segments 1–9 MLN
(4b) Species maceration	Male	Mouse, guinea pig (+rat, expt 2b)	7, 11 (young adult)	3 P 3 C	1 P 1 C	SI, proximal, middle, distal MLN

[*] Data for most of these animals were already reported in Smyth et al. (2005). Samples from one additional animal/group were collected with the 90 min group.
[a] P is particle fed; C is sterile double-distilled water fed control.
[b] SI is small intestine; MLN is mesenteric lymph node.

for each group was displayed as the bottom part of a stacked graph. For the relevant segment, the middle and upper parts represented the data for surface and luminal particle numbers, respectively. These latter figures were plotted as particle numbers/circumference section against intestinal length; unlike the AUCs, they took no account of section area, since this was not relevant to surface or luminal particles.

2.5. Maceration (experiments 2–4)

Specimens of proximal, middle and distal thirds of small intestine and of mesenteric lymph nodes were rinsed in 0.9% saline, placed in 15 ml of 15% potassium hydroxide at 60 °C for 2–3 days, stored at room temperature, diluted with 10 ml of 2% KOH and vortexed for 1 min prior to counting particle numbers in 20, 1 μl aliquots on glass slides (two per slide) covered with coverslips. Counting was completed within 2 months of sample collection. The number of particles/gram weight of tissue was calculated by extrapolating the average number of particles per 1 μl using a dilution factor of 25,000/weight of tissue, linking volume to weight.

2.6. Statistical testing (all experiments)

The Mann–Whitney *U* statistical test was used for detecting significant differences in segment uptake, reproducibility, uptake profiles and total uptake.

3. Results

3.1. Confirmation of model

The lack of respiratory distress and the visibility of yellow particles through the stomach wall confirmed the success of the gastrointestinal gavage. Control intestinal tissue showed no particles (data not shown).

Further substantiation of the soundness of the model came from the identification of particles in secondary sites such as lymph nodes, as well as in the wall of the intestine. Details of the uptake in these sites, illustrated in Fig. 1, and of other data confirming the soundness of the technique, are given below (Tables 2–7). The choice of 30 min as the time-point for experiments 2–4 was confirmed as appropriate by the fact that there is no significant increase in uptake from 30 to 90 min (Fig. 2; Table 2). Since intestinal dimensions varied across some groups with age and species, the data for these variables

Table 2
Average number of particles/circumference 14 μm sections/time

Time (min)	Compartment	Small intestinal section	
		2	8
5	Luminal	250 (85)	0
	Surface	66 (12)	0
	Tissue	42 (10)	0
30	Luminal	469 (157)	1 (<1)*
	Surface	114 (42)	0
	Tissue	85 (48)	<1 (<1)
90	Luminal	345 (20)	206 (75) $p = 0.03$*
	Surface	70 (15)	20 (5)
	Tissue	34 (3)	6 (<1)

Data are for proximal and distal segments of female rat small intestine for three time points, calculated from three sections. Values in brackets indicate standard errors. The data for 5 and 30 min are taken from 6 animals (Smyth et al., 2005): data for the 1 additional animal for each point were inside the range of the values in the table for all tissue, all surface and almost all luminal sites. Data for 90 min are new unpublished figures.

* Indicates significance between 30 and 90 min luminal data. No particles were found in sections from water fed control animals. Data are taken from experiment 1/time/microscopy (Table 1).

Table 3
Lengths (cm), section surface areas (mm^2) and particles/mm^2 (bold text) for all nine segments/age and species

	Surface area/particles	Small intestinal segment									SI length
		1	2	3	4	5	6	7	8	9	
Age											
3 weeks	Surface area	2.7	2.5	2.3	2.3	2.4	2.4	2.4	2.3	2.1	64.8
	Particles/mm^2	**71.5**	**225.4**	**250.5**	**162.6**	**19.2**	**2.5**	**0.3**	**0.0**	**0.1**	
7 weeks	Surface area	7.7	9.0	7.9	8.2	6.4	8.0	6.8	8.0	7.5	123.7
	Particles/mm^2	**74.3**	**52.8**	**77.3**	**57.1**	**30.6**	**9.0**	**8.1**	**0.4**	**0.1**	
17 weeks	Surface area	5.7	6.4	7.4	6.4	5.4	7.2	5.4	6.9	7.4	114.5
	Particles/mm^2	**9.3**	**15.3**	**30.5**	**27.3**	**75.2**	**0.5**	**0.0**	**0.1**	**<0.1**	
52 weeks	Surface area	5.2	5.8	6.0	7.3	6.0	6.4	5.6	7.5	9.1	122.8
	Particles/mm^2	**17.1**	**31.3**	**59.7**	**65.2**	**13.4**	**11.9**	**0.3**	**0.0**	**<0.1**	
Species											
Mouse	Surface area	3.4	3.2	3.9	3.4	2.9	3.4	2.9	2.7	2.4	49.3
	Particles/mm^2	**14.4**	**56.4**	**155.4**	**150.3**	**86.8**	**1513.3**	**126.5**	**141.6**	**159.4**	
Guinea Pig	Surface area	6.5	7.6	7.1	5.7	5.6	5.8	6.6	6.0	6.3	136.3
	Particles/mm^2	**10.8**	**58.2**	**49.8**	**33.9**	**35.8**	**57.7**	**37.0**	**37.3**	**22.7**	

No particles were found in sections from water fed control animals. Data are taken from experiment 3a/age/microscopy and 4a/species/microscopy (Table 1). The surface area measurements are used to convert the particle numbers/circumference section to particles/mm^2.

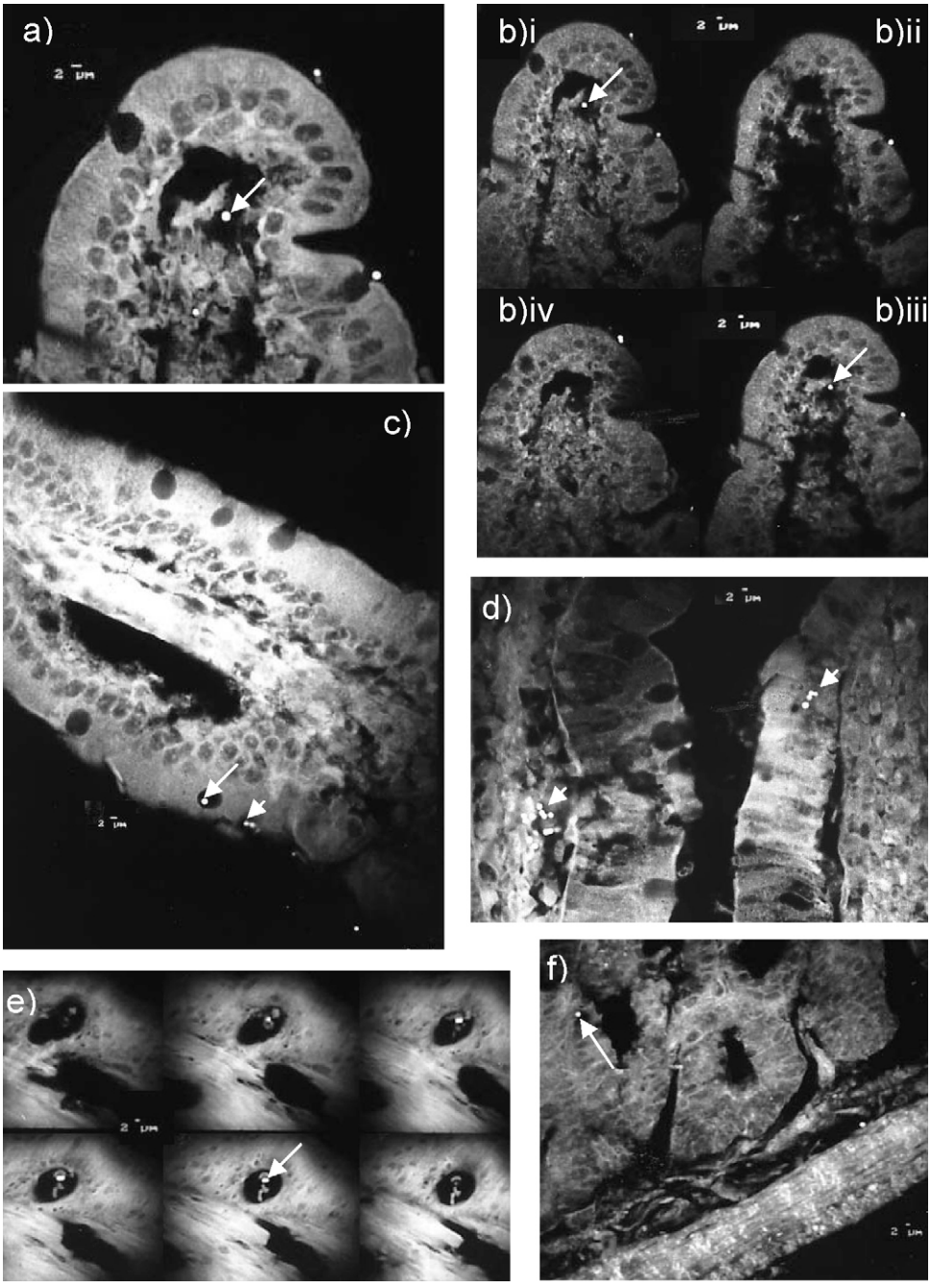

Fig. 1. Confocal images of propidium iodide stained cryosections of glutaraldehyde fixed small intestine of: (a) young adult male rat, 21 days old, showing villous tip with particles on surface and in the lamina propria, where the particle has caused a tissue tear during sectioning. (b) Young adult male rat, 21 days old, optical slices of the area seen in (a) showing that the particle is within the section, since it is not visible in the first and last levels of the series. (c) Young adult male rat, 21 days old, showing particles within a goblet cell and also at the surface or just inside the apical cytoplasm of another goblet cell. (d) Mid-villus region of 17-week-old rat small intestine, showing particles associated with enterocytes: more particles are visible within the lamina propria of the adjacent villus. (e) Female rat; optical slices showing presence of particle within the section, in a blood vessel in the outer layer of the muscularis externa; the diameter of the particle can be compared with that of the red blood cells within the vessel. (f) Young adult male rat, 21 days old, with one particle in the submucosa and another associated with cryptal epithelium.

include information on image analysis of intestinal sections as well as intestinal particle uptake (Table 3).

3.2. Age, gender and species

Variation in the size of the intestine in age groups was reflected in the range of dimensions for intestinal length or section area (Table 3) and of uptake profile (Fig. 3). Particles in the intestinal wall were most often seen in superficial, villous (Table 4), rather than deep tissues and a very small proportion was found in the Peyer's patches. The two younger age groups tended to have a relatively smaller percentage of particles in the deeper layers than the other two groups, but a relatively larger percentage in the Peyer's regions.

Table 4
Average number of particles/circumference in 14 μm of sectioned small intestine/age

Tissue site	Small intestinal segment (3 weeks/**7 weeks**/17 weeks/52 weeks)								
	1	2	3	4	5	6	7	8	9
Luminal (lumen and inter-villous)	423/**1050**/258/369	676/**923**/336/220	389/**1497**/1227/1167	665/**1482**/402/1361	2274/**734**/1325/472	178/**622**/102/925	1/**235**/3/35	0/**2**/0/0	1/**0**/0/0
Mucosal surface (enterocyte and goblet cell)	35/**46**/6/18	81/**48**/17/44	47/**41**/40/53	68/**58**/23/60	6/**25**/46/24	0/**2**/0/5	0/**6**/0/0	0/**0**/0/0	0/**0**/0/0
Villous regions (enterocyte, goblet cell and lamina propria)	16/**57**/5/9	52/**45**/8/20	47/**49**/20/32	30/**47**/15/38	5/**26**/31/9	1/**6**/0/5	0/**6**/0/0	0/**0**/0/0	0/**0**/0/0
Cryptal and deep regions (cryptal epithelia, pericryptal stroma, submucosa, blood vessels, muscularis and serosa)	1/**2**/0/1	4/**4**/1/1	7/**10**/2/4	6/**4**/2/6	0/**2**/9/3	0/**1**/0/2	0/**1**/0/0	0/**0**/0/0	0/**0**/0/0
Peyer's patches (FAE and lymphoid tissue)	0/**0**/0/0	0/**0**/0/0	2/**2**/0/0	1/**2**/1/0	0/**0**/0/0	0/**0**/0/0	0/**0**/0/0	0/**0**/0/0	0/**0**/0/0

Data are for 3-week/7-week (bold text)/17-week/52-week-old male rats ($n = 6$), for all nine small intestinal segments 30 min after particle administration, calculated from 10 sections. Standard error of means is not included as these data are composite figures. Mean small intestinal length for males of 64.8 ± 2.6 cm (3 weeks); 123.7 ± 10.8 cm (7 weeks); 114.5 ± 6.5 (17 weeks); 122.8 ± 5.6 (52 weeks). No particles were found in sections from water fed control animals. Data are taken from experiment 3a/age/microscopy (Table 1).

Table 5
Variation in dimensions (length, cm, and section area, mm^2) and uptake measurements (total uptake; AUC, *i.e.* particle numbers/section area/intestinal length; percentage uptake)/age and species

Group	Subgroup age	Length	Section area	Total uptake for segments 1–9 taking account of intestinal length	AUC tissue uptake (particles/mm^2 summed for small intestine segments 1–9)	Percentage uptake
Age (rat)	3-week young	**3 < 7**, **17**, **52**	**3 < 7, 17, 52**	0.88×10^6	0.34 **3 > 17, 52**	0.04
	7-week young adult		**Seg 1, 7 > 17** **Seg 1–3, 7 > 52**	2.52×10^6 **7 > 3, 17, 52**	0.26 **7 > 17, 52**	0.13 **7 > 3, 17, 52**
	17-week mature			0.86×10^6	0.12	0.04
	52-week old			1.24×10^6	0.16	0.06
Species (young adult)	Mouse	Mouse < rat, guinea pig	**Mouse < rat**, **guinea pig**	2.50×10^6	0.74	0.32
	Rat		**Seg 4, 6, 8 rat > guinea pig**	2.52×10^6	0.26	0.13
	Guinea pig	Guinea pig > mouse, rat		2.44×10^6	0.31	0.12

Statistical testing was carried out within all groups, significant differences are shown as bold text: seg is segment. No particles were found in sections from water fed control animals. Data are taken from experiment 3a/age/microscopy and 4a/species/microscopy (Table 1).

Table 6
Average number of particles/circumference in 14 μm of sectioned small intestine/gender

Tissue site	Small intestinal segment (**male**/female)								
	1	2	3	4	5	6	7	8	9
Luminal (lumen and inter-villous)	**1050**/947	**923**/786	**1497**/3779	**1482**/2100	**734**/1219	**622**/494	**235**/171	**2**/84	**0**/0
Mucosal surface (enterocyte and goblet)	**46**/69	**48**/125	**41**/88	**58**/50	**25**/59	**2**/10	**6**/8	**0**/1	**0**/0
Villous regions (enterocyte, lamina propria and goblet)	**57**/59	**45**/66	**49**/85	**47**/39	**26**/41	**6**/5	**6**/5	**0**/1	**0**/0
Cryptal and deep regions (cryptal epithelia, pericryptal stroma, submucosa, blood vessels, muscularis and serosa)	**2**/11	**4**/16	**10**/13	**4**/8	**2**/11	**1**/3	**1**/0	**0**/0	**0**/0
Peyer's patches (FAE and lymphoid tissue)	**0**/1	**0**/0	**2**/3	**1**/2	**0**/2	**0**/1	**0**/1	**0**/1	**0**/1

Data are for male (bold text)/female rat ($n = 6$), for all nine small intestinal segments 30 min after particle administration, calculated from ten sections. Standard error of means is not included as these data are composite figures. Mean small intestinal length for males of 123.7 ± 10.8 cm and 94.2 ± 3.6 cm for females. No particles were found in sections from water fed control animals. Data are taken from experiment 2a/gender/microscopy (Table 1).

Table 7
Particle numbers per μl of macerated sample for small intestine and mesenteric lymph nodes/age, gender and species

Region	3-week-old male rat	**7-week-old** male young adult rat	17-week-old male rat	52-week-old male rat	Female young adult rat	Mouse male young adult	Guinea pig male young adult
Proximal SI	1.9×10^7	$\mathbf{7.1 \times 10^6}$	2.0×10^6	3.3×10^6	1.8×10^6	2.2×10^6	2.3×10^5
Mid SI	7.7×10^6	$\mathbf{2.3 \times 10^7}$	1.2×10^7	1.9×10^7	3.3×10^6	4.1×10^6	5.3×10^5
Distal SI	2.7×10^5	$\mathbf{4.6 \times 10^5}$	9.3×10^4	2.4×10^5	1.2×10^4	2.3×10^7	2.5×10^5
MLN	3.2×10^4	$\mathbf{3.2 \times 10^5}$	6.5×10^5	5.5×10^3	1.3×10^5	3.0×10^4	7.5×10^3
Total SI (particle/μl)	2.7×10^7	$\mathbf{3.1 \times 10^7}$	1.4×10^7	2.2×10^7	5.1×10^5	2.9×10^7	1.0×10^6
Percentage relative to administered dose (%)	1.6	**1.8**	0.8	1.3	0.3	4.3	0.1

SI is small intestine and MLN is mesenteric lymph node. Percentage of particles taken up relative to administered dose is also given for each group. No particles were found in sections from water fed control animals. Data are taken from experiment 2b/gender/maceration; 3b/age/maceration; 4b/species/maceration (Table 1).

The young adult group (7 weeks) showed significantly greater total uptake ($p = 0.005$) and percentage uptake ($p = 0.05$) than both the younger and the older group (Table 5). The youngest group (3 weeks) had the shortest and also the thinnest ($p \leq 0.0005$) intestinal dimensions of all the groups. The intestine in the young adult group (7 weeks) was also thicker proximally than that in the two older groups, for segment 1, thicker than the 17 weeks group ($p = 0.0001$) and for segments 1–3, thicker than the 52 weeks group ($p \leq 0.004$). When these figures for intestinal thickness were taken into account, the AUCs for uptake profile for the two younger groups were significantly higher ($p \leq 0.036$) than those for the two other groups (Fig. 3; Table 5).

Uptake in female animals tended to be higher than in males (Table 6), but this was only at the level of a strong trend ($p = 0.06$). The distribution of particles in superficial, deep and Peyer's patch regions appeared to be similar to that in the males, although a slightly greater percentage of particles was detected in the deeper regions and Peyer's patches of the female rats (Table 6).

There was no difference across species in total uptake and percentage of administered dose (Table 5). Although the wall of the mouse intestine was significantly thinner than rat and guinea pig in all nine segments ($p \leq 0.008$) and rat was thicker than guinea pig in segments 4, 6 and 8 ($p \leq 0.002$), there was no significant difference in AUC and therefore uptake profile for the three species, despite the marked difference in the shape of the mouse curve (Fig. 4; Table 5). The distribution across various compartments was similar across the three species, although in the mouse group there was a greater percentage in deeper than in superficial regions (data not shown).

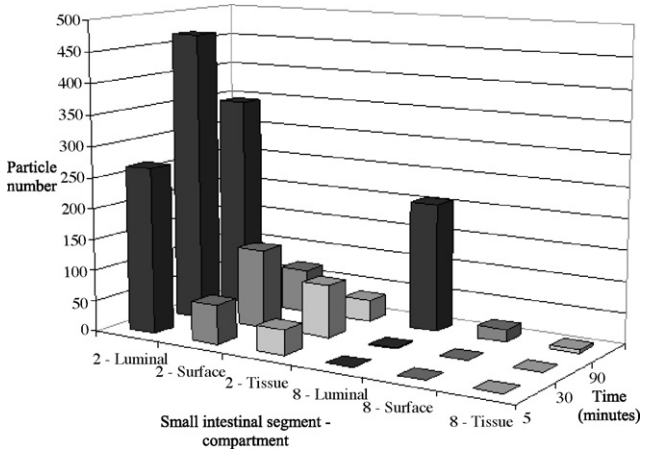

Fig. 2. Particle numbers 5, 30 and 90 min after administration in luminal, surface and tissue compartments for small intestinal segments 2 and 8.

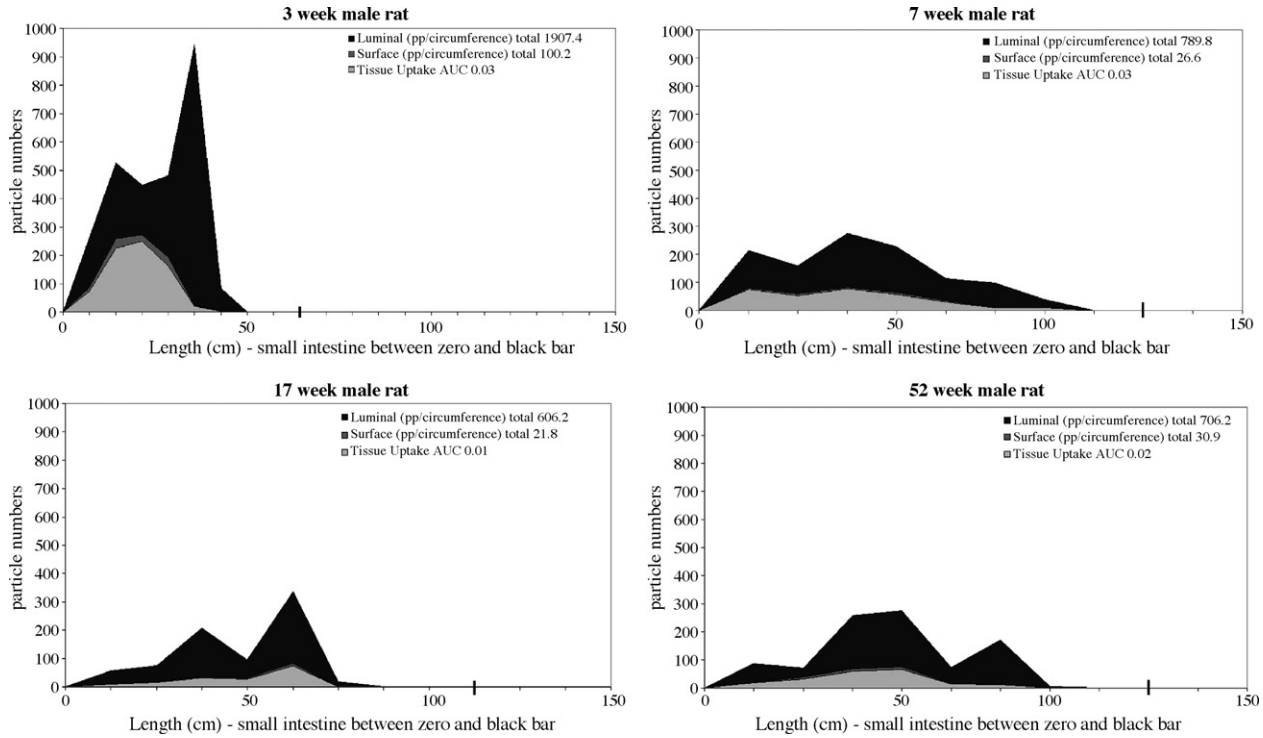

Fig. 3. Stacked uptake profiles for four ages of male rats. The vertical line (|) marks the end of the length of the small intestine, with *X*-axis subunits representing segments 1–9. Total particle numbers for surface and luminal compartments are provided as particles/circumference (14 μm section) and AUC value is for tissue uptake in particles/mm^2 all summed for segments 1–9.

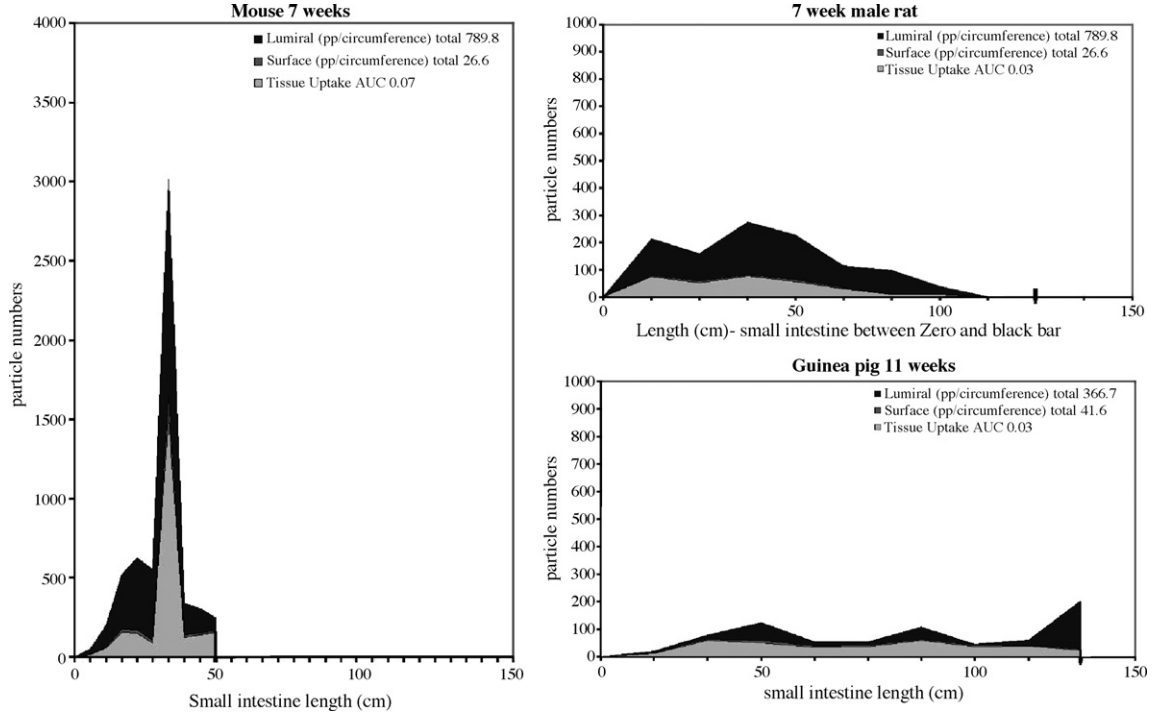

Fig. 4. Uptake profiles for three species of young adult males. The vertical line (|) marks the end of the length of the small intestine, with *X*-axis subunits representing segments 1–9. Total particle numbers for surface and luminal compartments are provided as particles/circumference (14 μm section) and AUC value is for tissue uptake in particles/mm^2 all summed for segments 1–9.

In all groups, particle uptake through the small intestinal wall and onwards to mesenteric lymph nodes was confirmed by maceration (Table 7).

4. Discussion

Past estimates of the extent of particle uptake have varied by several hundred fold, prompting O'Hagan (1996) to call for more reliable data. Since these variations are largely due to differences in experimental methodology, it is important to begin this discussion with a detailed consideration of the methods used in the current study. This will then be followed by a section on the extent of particle uptake and how this is affected by variables such as gender, species and age.

4.1. Methodology

The basic soundness of this model (Smyth et al., 2005) was confirmed by the lack of evidence of respiratory distress, the absence of particles in control tissue and the presence of a fluorescent bolus or particles in the gastrointestinal organs of experimental animals at post-mortem. Movement onwards from the intestine is shown by the presence of particles in secondary sites, such as lymph nodes. The use of 30 min as the time point for cross comparisons of age and species is supported by the data for 5–90 min presented here (Table 2; Fig. 2) and also by earlier findings (Hodges et al., 1995a). The locations of the particles are confirmed by confocal microscopy, showing that they are within the thickness and not on the surface of the sections, which could indicate carry-over from an intraluminal position.

The *in vivo in situ* nature of the model, simulating as far as possible the human situation, would suggest oral administration of particles as the preferred method. However, previous concerns about subsequent particle loss through the mouth led to the use of gavage to the stomach, where animal size made this possible. Since there was no significant difference in particle uptake for proximal small intestine 30 min after oral administration as opposed to gavage (Carr et al., 1996a), the latter could be regarded as an adequate substitute for the former.

Although microscopy techniques give detailed information on the exact location of particles, total tissue numbers have also been estimated using established procedures for maceration of bulk tissue (LeFevre et al., 1989; Ebel, 1990; Hodges et al., 1995b). This is particularly useful for secondary sites such as mesenteric lymph nodes (McMinn et al., 1996). Care was taken to ensure that maceration counts were done in groups, within the first 2 months, since, irrespective of the light level of storage conditions, exposure to the macerating fluid led to particle fading, particularly in the first month (Carr et al., 1996b). This was not seen in microscopy sections (unpublished data), confirming this technique as the method of choice. This requirement to select an assay method to optimise the accuracy of particle quantification also highlights the fact that the current study deals with only one type of particle/tissue interaction. Further work is needed across a range of particle types, allowing discussion of the parameters affecting this, such as particle size or charge; hydrophilic or toxic properties; ability to be loaded with pharmacological agents.

4.2. Extent of particle uptake and effect of variables

The finding that the uptake is almost entirely villous in this *in vivo in situ* model, rather than just associated with lymphoid tissue, confirms previous reports over a period of 10 years (Hodges et al., 1995a; Smyth et al., 2005).

Percentage uptake was low, but in line with some literature reports. The bulk tissue values of 0.1% (guinea pig) to 4.3% (mouse) at 30 min after administration matched fairly well with values of 0.01% (Ebel, 1990) to 3.6% (Jani et al., 1990). The smaller range (0.04–0.32%) of uptake obtained from microscopy data reflected the higher resolution of this technique and its ability to exclude from counting all luminal and surface particles. This figure is almost certainly an underestimate, since it only applies to 30 min specimens. At this time point the particles counted are almost certainly more recently taken up than those which entered at 5 min (Hazzard et al., 1996), many of which may already have reached the serosa prior to moving on to secondary organs. In relation to drug delivery, this small value for percentage uptake further highlights the need for the identification of a binding agent that would enhance microparticle uptake. In relation to environmental toxicology, on the other hand, particle distribution may not be uniform and environmentally hazardous particles may be concentrated in small regions, thereby affecting local microdosimetry.

In this study, the animals of different ages and species have intestines of widely different dimensions. This called for the addition of image analysis to the experimental model, since the description of uptake as a percentage of the administered dose, although useful, would mask the many variables involved. However, the uptake profiles provide a satisfactory summary of the overall situation; adding the data for proximodistal distribution of luminal and surface particles to the AUC graphs puts the tissue uptake into an anatomical context.

The findings of these experiments may be divided into two groups. The first relates to host-associated variables that do not appear to affect the extent of uptake and the second relates to those which do. In male and female rats, there is some variation in the length, but not in the thickness of the wall and there is no substantial variation in uptake. This is less at variance with the earlier finding of higher uptake in females 30 min after gavage than appears at first sight, since, although the level of significance is different, the trend here ($p = 0.06$) is in the same direction as the published value ($p = 0.05$; Smyth et al., 2005). The lack of information on the oestrous cycle of the females is a weakness, since hormone status may be responsible for the increased uptake in late pregnancy and early lactation (Smyth et al., 2005). Species differences did not substantially affect AUC tissue uptake or the percentage uptake of administered dose, despite the variations in intestinal length and thickness of the wall. Comparisons with the literature are difficult, since the lesser uptake by guinea pig than rat (Harrison and Fritsch, 1992) is for younger animals, while the greater uptake by rat than rabbit (McClean et al., 1998) referred to only one part of the ileum, as well as to a different species and

particle type. From the data presented in the current paper, cross-species variation therefore appears to be minimal, at least in the sensitive young adult population. This is of potential clinical and environmental importance; although extrapolation of these *in vivo in situ* animal data to the human species is of debatable validity, the uptake of similar particles could be in the order of 0.12–0.32% of the administered dose.

In contrast, the age of rats does affect the extent of uptake. The youngest group, 3 weeks, has high AUC tissue uptake, despite also having the shortest and thinnest intestine and therefore less tissue to hold particles in the sections examined. The AUC tissue uptake of the young adult 7-week-old group is not significantly different, despite its thicker-walled and longer intestine (Penzes and Skala, 1977; Mandir et al., 2005). However, the percentage of administered dose taken up is higher in this young adult rat group than in the other ages studied. Further increase in age was associated with thinner proximal segments and decreased uptake. This suggests that younger individuals are more at risk during exposure to particulate pollutants. In addition, an increased uptake at this age could have implications in drug delivery therapies. Further work should be carried out to determine whether this increased level of microparticle uptake is affected by the more reactive immune system and higher metabolic activity at this age. Although this conclusion is in line with some reports (Harrison and Fritsch, 1992; Seifert et al., 1996), others have described a decrease (LeFevre et al., 1989) or no variation with age (Simon et al., 1994). The decreased uptake in older groups is important in itself, since changes in intestinal motility have already been highlighted for such individuals (O'Mahony et al., 2002). While the reduced uptake would help to limit the amount of toxic material taken up, it could also make drug delivery by loaded microspheres less efficient.

The fact that young adults may be most 'at risk' is of concern, since this group is also reproductively active; late pregnancy and early lactation are associated with further increases in uptake (Smyth et al., 2005). Epidemiological evidence of vCJD risk suggests that small intestinal changes during childhood growth could contribute to the 'strikingly' high risk of adolescents contracting vCJD (Boelle et al., 2004). Although prions are orders of magnitude smaller than the microparticles used in this study, the importance of their passage allows vCJD to be included in the list of possible applications of unconventional changes in the permeability of the small intestinal mucosa. This could be linked to age-related variations in basic intestinal biology, such as the increase, peaking and subsequent decrease in mitotic activity and apoptosis (Mandir et al., 2005). Their conclusion, that cell proliferation rather than crypt fission is important in small intestinal development, points to the need to compare directly the age-related variation in microparticle uptake with these other cellular parameters. The subcellular level is also important, since there are issues to be resolved between microfold (M) cell uptake at Peyer's patches (Ermak et al., 1995; Jepson et al., 1995; Thomas et al., 1996; Beier and Gebert, 1998) and tight junction unzipping to allow the passage of particles, as proposed by other groups (Bjork et al., 1995; McCullough et al., 1995; Wiesner et al., 2002). The proteins involved in these junctions can now be identified (Lapierre, 2000; Gonzalez-Mariscal and Nava, 2005) and changes here are relevant to the paracellular routes of passage that this implies.

In conclusion, the quantification method employed here allows comparisons across groups, especially where variations in intestinal dimensions complicate the situation, as in cross-species studies. These techniques provide a potentially sounder basis for the extrapolation of data from smaller species to humans and indicate that young adults may have higher uptake than both younger and older age groups. This may have dosage implications in relation to pollution, drug delivery systems and disease pathogenesis such as organ failure and vCJD: further work is needed on the uptake characteristics of this sensitive subpopulation.

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