

A summary and update on the 'House of Lords Science and Technology Select Committee Call for Evidence: Nanotechnologies and Food'

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Introduction

When cells fail to recognise surface molecules or molecular structure of small particles, the fate of these particles may be determined by their physical size and no longer by their chemical composition. Nanoparticulate, nanosized and nanostructured are then descriptors that relate to a dominant characteristic.

Natural Exposure

The human gut has been exposed to non-biological particles of varying sizes for millennia. For example, dietary ferritin is a small nanoparticle (●) of 13 nm diameter when whole and 2.5 nm as the smallest core sub-unit, while dust and soil nanoparticles tend to be hundreds of nm in diameter/length (●). Four uptake (absorption) mechanisms have been proposed in the gastrointestinal tract (Figure 1):

1. Through 'regular' epithelial cells (gut-lining cells) via a route termed endocytosis ('engulfing' the particle). Very small particles- tentatively generally < 20nm in diameter.
2. M cell uptake (transcytosis) at the surface of intestinal lymphoid aggregates. This is the quintessential pathway for gut particle uptake and is very well described, especially for large nanoparticles (≥ 100 nm), although smaller particles are also likely to be able to access this route. M cells have a 'surveillance' role in the gut and are specialised in particle uptake.
3. Persorption. Volkhemer's concept of passage through 'gaps' at the villous tip following loss of enterocyte(s) to the gut lumen. Small and large nanoparticles potentially access this route but its quantitative validity is unclear.
4. Putative paracellular (between cell) uptake. Generally junctional complexes are unlikely to allow even the smallest of nanoparticles to permeate but certain drugs and/or dietary situations, and especially diseases, may alter this situation allowing influx of very small nanoparticles. Theoretical pathway as it stands.

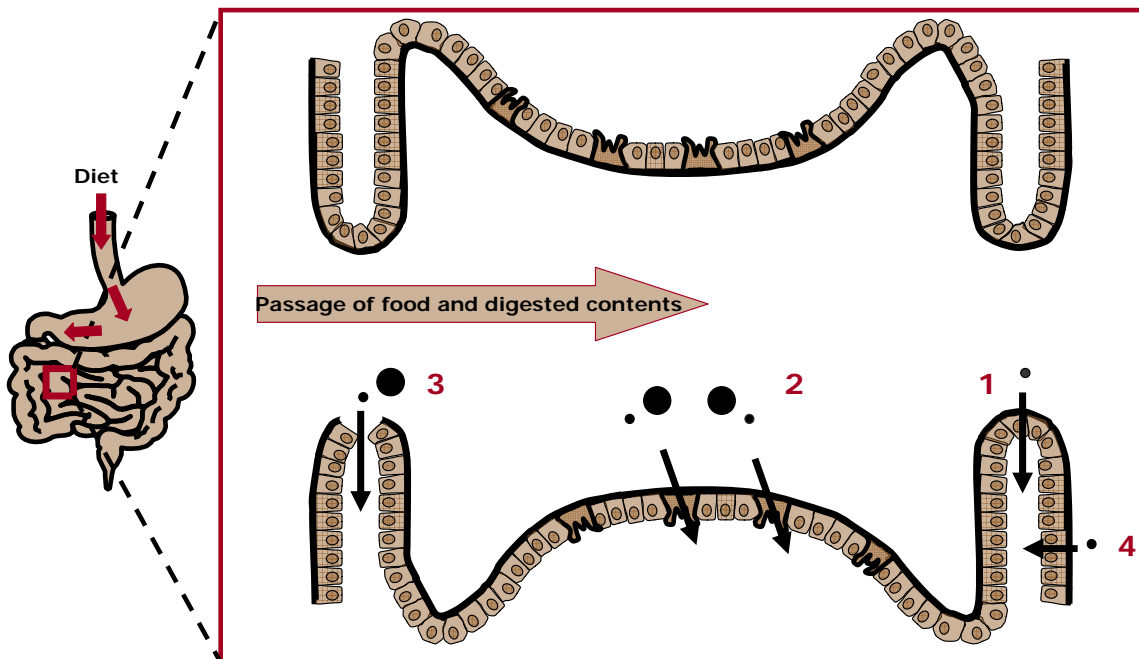


Figure 1: Schematic representation of different routes for particle uptake in the small intestine. The numbers refer to those pathways described in the text. Uptake via (1) regular epithelial cells (2) M cells of the lymphoid aggregate (3) persorption and (4) the theoretical paracellular pathway.

Regardless of mechanisms, it is clear that ingested particles across the nano-range (0-1000 nm) will be absorbed to some extent into both the circulation and the gut tissue itself. Percentage absorption will depend on many factors (e.g. size, surface charge, host gut permeability, etc). But even if only 0.1% of a total 10^{13} ingested particles is absorbed, that corresponds to 10^9 particles absorbed/day.

From the circulation, particles will be retained by cells in the liver and other vascular organs. From the gut tissue, cells can migrate systemically with their cargo (e.g. particles), especially to mesenteric lymph nodes. The persistence or degradation of particles at any site depends upon the physico-chemical characteristics of the particles but even undegradable particles have some clearance through cellular-shedding in the gut and lung.

Man-made Particle Exposure – Currently

Silicates, aluminosilicates, titanium dioxide and carrageenan are among the typical man-made, or at least man-modified, particles that the human gut is now exposed to,

especially in the Western world, on a daily basis. Exposure has been for decades-as food additives mainly. Except at MRC-HNR there is little research on the gut-associated effects of these although some appear to accumulate in gut tissue. Nonetheless, studies to-date suggest that, overall, these particles are safe and even if they can be shown to have any adverse effects it will almost certainly be in a small minority with a different genetic make-up. However there is no evidence for this currently.

The above particles are almost all in the larger nano-range (being ≥ 100 nm diameter/length). There is, in the UK, no evidence currently for the significant intake of new/man-made small nano-sized particles, although, increasingly at the global level, proposals for this are made in industry and in research studies.

Man-made Particle Exposure – Future

'Nanosizing' can have a variety of commercial advantages for certain foods, supplements (especially), medicines, food packaging and other materials that may be ingested. However, in many cases, the 'nanosized' foods will undergo simple gastrointestinal digestion prior even to meeting any cells (Figure 2). Examples include 'nano-salt' (1) and probably some 'nano-micelles' (2). However, even with nano-micelles that are absorbed whole, they will undergo fairly rapid cellular degradation and are likely to be recognised for their molecular structure rather than their nanosize. Indeed it should be noted that yoghurt and milk are foods containing nano-micelles (40-300 nm) of casein that occur in large abundance in the intestinal lumen upon ingestion. For competitive commercial reasons, as well as the potential to lose scientific/toxicological focus, it would seem sensible that such foods are considered separately with regards to further 'nano-legislation'.

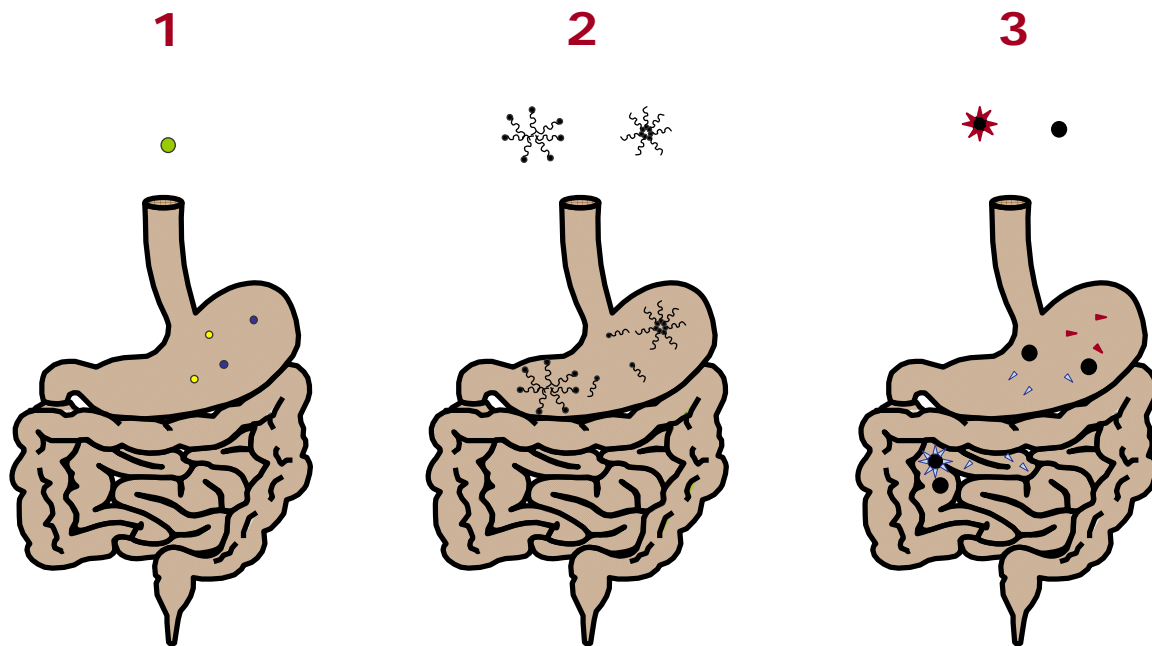


Figure 2: 1, Some nanoformulated materials, e.g. nanosalts, are likely to be digested in the gut before any cellular exposure. 2, Micellar nanoformulations may partially degrade in the gut or be absorbed whole, but are likely to be rapidly broken down in cells. 3, In contrast, truly or transiently persistent nanoparticles are likely to lose any surface adsorbed material in the stomach, but may themselves remain intact, and then, later in the gut, could (depending on size, surface charge etc.) adsorb other soluble luminal molecules before cellular uptake.

Thus, in the case of micellar nanoparticles it is highly likely that the constituent molecules would dictate toxicity, rather than their aggregated nature to form a nanomicelle, although this latter property could influence bio-distribution.

In the final scenario in Figure 2, novel nanoparticles may be bio-persistent, either transiently as there is gradual cellular breakdown, or truly persistent as they can only be cleared with the sloughed cells, as noted above. If the latter process is slower than the rate of uptake then particles may accumulate. Examples could *speculatively* include, nano-silver, nano-clays and nano-silica. Depending upon their size, surface charge etc., ingested particles may adsorb (to their surface) other soluble molecules, including bacterial toxins, from the gut lumen, and carry these across into cells (Figure 2:3). Probably the larger nanoparticles are better at this.

Particle Toxicity: Factors and Why Nanoparticles?

A number of poorly predictable properties dictate particle toxicity- e.g. crystalline structure, surface reactivity, dissolution characteristics, adsorptive properties etc. So, for example the α -quartz form of silicon dioxide is a toxic particle while the amorphous form of silicon dioxide is not. A second example, mediated by a similar process to that of

quartz, is that nano-particulate hydroxyapatite may be toxic to cells while some other forms of nano-particulate calcium phosphate are considered less so.

Particle shape can also affect particle toxicity. Thus asbestos, erionite and some man-made nanotubes appear toxic due to their high aspect-ratio or 'needle-like' shapes.

Finally, size. This is often poorly understood. The large majority of particles are fairly inert/non toxic unless they have some specific property, as noted above. In the absence of any 'special property', particle toxicity can be considered in two simple forms:

(1) Direct toxicity. Normally mediated through 'free radical' activity and, in this case, smaller particles are considerably more active than the same mass of larger particles. This appears to be a surface area phenomenon. However, just because this can happen, we must ask does it happen? Many experiments use such unrealistic particle doses that extrapolation to lower doses, that represent real exposures, may be artefactual. The result of drinking a bottle of whisky one evening tells us little about the result of one drink per evening over a few months. Secondly, most tissues, including the gut and circulation, are armed with complex and replenishable antioxidant defences to combat such acute (short-term) exposures. In doing so, however, there may be downstream costs (long-term).

Accumulating evidence suggests that lung exposure to nanoparticles is linked with an increased risk of chronic cardiovascular disease. A second potential lesson from the lung is that certain individuals (e.g. those with asthma) can experience an exacerbation of disease upon acute exposure to an abnormally high environmental dose of particles (e.g. at peaks of urban pollution). However it is the view of these authors (but not the wider community) that this latter phenomenon, as opposed to the chronic systemic effects, could be more related to the large nanoparticle fraction (●) than it is to the small fraction (•), leading onto the second potential mechanism of general particle toxicity.

(2) Large nanoparticles (or aggregated small ones) can make good cellular 'adjuvants' such that an immune response to a protein/allergen/antigen is enhanced or 'polarised' when exposure is in the presence of a particle. Contact between the allergen/antigen and the particle (e.g. adsorption) appears important.

What is Special about Nanoparticles?

Three things. First, as detailed above, in the absence of a 'special property' for particle toxicity, all particles will be more directly toxic to cells as small nanoparticles than as

larger ones. The pros and cons of this observation are noted above. Secondly, as a rough guide, particles < 100 nm diameter will be taken up by cells through a different pathway to that of larger particles (Figure 3), meaning that they will access different cellular compartments and have different cellular effects. Again, 'induction of free radicals' versus 'adjuvant activity' are the basic differing outcomes. Thirdly, very small nanoparticles are especially mobile and motile and may access all areas of the body including even the brain and all areas of the cell including even the nucleus (being smaller than nuclear pores). It is this latter property that probably makes very small nanoparticles most worrisome to scientists and hence the translation of this concern (but not the knowledge of why) to the public.

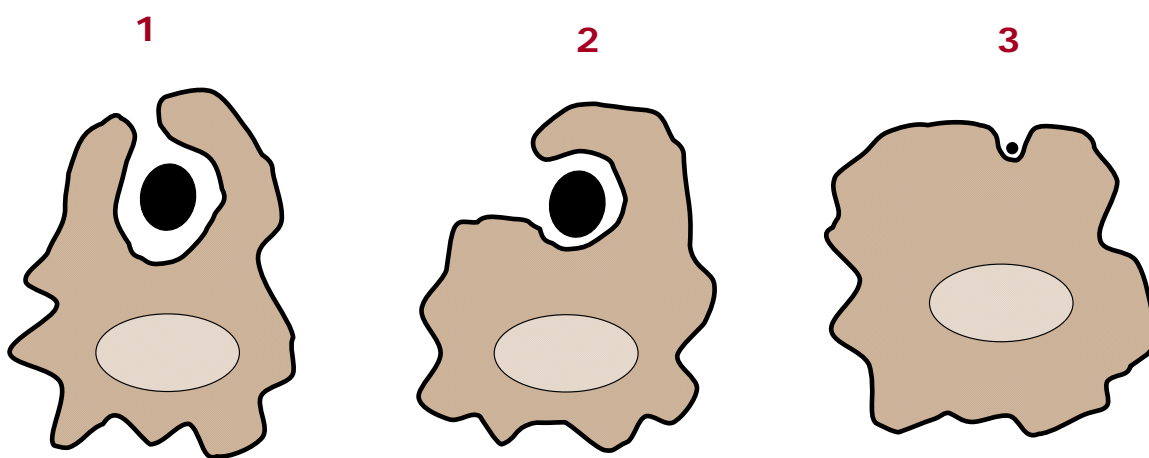


Figure 3: Schematic representation of cellular particle uptake for large particles via **1.** active phagocytosis or engulfing of large particles and **2.** macropinocytosis which is a different type of active particle capture. These events are triggered by the size of the particle. **3.** small particles are taken up by constitutive pinocytosis and are processed by the cell in a different fashion.

Finally, it should be noted that in the absence of specific particle toxicity there is no logical reason to assume that, in the gut, smaller nanoparticles will always have worse adverse health effects than larger ones or that either will have any adverse health effects at all. It will depend on many other variables including host genotype, persistence, dose, and ability to adsorb gut luminal molecules. And thus there is no logical reason to use 100 nm as a cut-off for adverse effects, even though, as discussed, this size discrimination may help determine the type of cellular effect.

Other Important Factors

- 1) Particles may aggregate so that their behaviour during at least part of the exposure process is more typical of large particles (●) even though their single unit is as a small particle (•). This is especially true for small nanoparticles.
- 2) Particles are rarely seen by cells in their 'native form'. Most particles readily adsorb to their surface molecules and ions from their environment. In the gut, particle surfaces may be 'cleared' in the acid and enzyme-active area of the stomach but re-adsorb material further down the G.I. tract. In this environment, bacterial proteins and carbohydrates are especially common.
- 3) Classical toxicity or toxicology studies may be poor or even misleading at deciphering particle toxicity following oral exposure. In particular, long-term (decades) effects and host genotypes cannot be mimicked in animal studies. Instead a 'logic algorithm' and some targeted *in vitro* tests may be more useful.
- 4) Nanotechnology may actually serve to make some materials less toxic. For example, MRC-HNR is developing a transiently stable nano-formulation of supplemental iron which should exhibit much less toxicity to the intestinal mucosa, and therefore side-effects, than the current common therapeutic supplements, namely ferrous sulphate and other ferrous salts.

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