Assessing the toxic risks of the nanotechnology industry

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Abstract

This article analyses the risks facing the nanotechnology industry due to the toxicity of some nanomaterials. The emphasis is on brevity while being comprehensive in coverage.

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1. Introduction

The literally toxic risks faced by the nanotechnology industry are predominantly derived from the toxicity of certain of its products. We can confine attention to those sectors of the industry that manufacture nanomaterials; that is, nano-objects and nanostructured materials. In other words, we can leave aside the important but commercially much smaller nanometrology (i.e., instrumentation) sector and the very large semiconductor processing sector. The activities of the former pose negligible risks, and the risks associated with the activities of the latter are, essentially, the same as those that were already present before the component feature size of very large scale integrated circuits fell into the nanoscale. We can also leave aside the "grey goo" scenario, in which nanoscale assemblers building structures atom-by-atom and first of all making copies of themselves in order to ensure an adequate volume of production end up doing nothing but self-replication, consuming all the Earth's material resources in the process [66], since assemblers are still in a very primitive stage of development [59].

The only industrially important nanostructured materials at present are those consisting of nano-objects (particles, fibres or platelets) incorporated in a matrix (e.g., polymers and metals). Globally, quite a few companies are producing nano-objects on a more or less commercial basis and typically they are selling these primary materials to other companies, which may already have a long history in producing matrix materials, for creating nanocomposites. The purist might argue that the nanocomposite is not a true nanomaterial because the nano-objects' positions are typically only determined statistically rather than with atomic precision, but one can consider such materials to be at least an approximation to a true nanostructured material.[1, 2]

1.1 *Evaluating risk*

Our subject is, therefore, the risks associated with the production and use of nanomaterials, as far along the supply chain as the manufacturers' responsibility can reasonably be said to exist. The fundamental equation describing the risk derived from coming into contact with substances is commonly presented as:

$$\text{Risk (from the substance)} = \text{Exposure (to the substance)} \times \text{Hazard (from the substance)}. \quad (1)$$

The fact that we can write such an equation implies that all the terms can be quantified. Unfortunately "exposure" is ambiguously defined. In everyday language, we might simply say that we have been "exposed to light", or "exposed to a chemical", but as soon as the exposure is

[1] Besides, the European Union's definition of nanomaterial is that it is "a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm" [11], clearly covering statistically ordered nanocomposites.[2]

[2] Definitions: an agglomerate (can also be termed a secondary particle) is a collection of weakly bound primary particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components; the forces holding aggregates together include covalent bonds, or those resulting from sintering or complex physical entanglement such that the aggregate is indivisible [24].

quantified it is clear that it is the integral of the irradiance (for light) or partial pressure (for a vapour) over the (time) interval during which the exposure takes place. Irradiance being expressed in the number of photons falling on unit area per unit time, given the exposure interval one can precisely calculate the number of photons falling on a photographic plate, for example. Chemicals pose more difficult challenges; indeed the case for an "exposure science" has recently been put forward [33]. The official "Workplace Exposure Limits" (WELs) [10] are defined as "concentrations of hazardous substances in the air, averaged over a specified period of time referred to as the time-weighted average (TWA)". Thus, for example, the long-term exposure limit (8-hour TWA reference period) for fused respirable silica dust is 0.08 mg/m^3 (*loc. cit.*, p. 32). Knowing the average rate of human respiration (expressed as a certain volume of air per unit time), this can immediately be expressed as a certain mass of silica, which is the actual dose received by the exposed subject.

Hazard may be expressed in various ways. Very often, laboratory animals are given increasing doses of a (suspected) poison under controlled conditions and the proportion of animals dying within an appropriate interval following administration of the poison is recorded. From this data a dose–response curve can be constructed. Thus, hazard (with respect to lethality) is expressed as the probability of lethality per dose, multiplying with which, according to equation (1), gives risk as the probability of lethality. The information provided by the dose–response curve is sometimes compressed into a single number, such as the lethal dose for 50% of the animals, commonly represented as LD_{50}. If the curve rises very steeply at around the LD_{50}, it might be appropriate to express the risk in binary fashion; for a dose less than LD_{50} the risk is zero and above it, one. (Instead of lethality, the hazard could be expressed as the probability of contracting a certain disease.) In general, though, it is more satisfactory to convolute the actual exposure, fluctuating, possibly quite significantly, during the interval under consideration with the dose–response curve. This is more sophisticated than the approach recommended in [10], which also takes no account of the body's ability to eliminate toxins with which it has been dosed. That, in turn, depends on other factors including the subject's genetic constitution, recent dietary intake, bodily activity, general level of fitness, exposure to other toxins etc. All of these could be incorporated into an expanded equation (1) but such sophistication is scarcely justified by the present level of knowledge of the toxicity of nanomaterials. Here, we merely aim to characterize the hazards from nanomaterials qualitatively, while pointing out how it could be done more quantitatively. Likely exposures will be estimated for the general public and in the factory and will be compared with natural exposures to nanomaterials.

The WEL approach is rooted in the concept of "no observed adverse effect level" (NOAEL) or "lowest observed adverse effect level" (LOAEL) [10], which implies a threshold exposure level (i.e., concentration in the atmosphere), below which there is no hazard no matter how long exposure continues. It is, however, recognized that there are substances for which there may be no threshold, in particular DNA-reactive chemicals, exposure to which at any level may ultimately cause cancer, although only decades after exposure, hence causation is very difficult to demonstrate. In general, therefore, there is no threshold, from which it follows that the only way to eliminate risk is for exposure to be zero.

1.2 *Evaluating the reasonable cost of measures to mitigate risk*

Practically speaking, that is impossible: the very fact that a substance is commercially traded implies that there is a beneficial use for it. No matter how stringent the precautions, accidents may occur and, hence, unless the hazard is zero there must always be some risk. Risk must be traded against benefit; this is a familiar enough concept, applicable even in prehistoric times: breathing and eating are essential, but the presence of oxygen in the body and the oxidative reactions associated with the digestion of food create reactive oxygen species, which cause mild inflammation. Oxygen and food are hazardous but indispensable. A similar situation obtains with many, perhaps all, of those artefacts called "modern conveniences": many would consider motoring to be an indispensable part of life nowadays, yet it is responsible for large numbers of fatalities every year. Equation (1) can only, therefore, be part of the story. The risk can be quantitatively set in the context of overall life by making use of the life quality index Q [47, 62], a composite parameter incorporating annual income, work–life balance and life expectancy of (typically groups of) individuals:

$$Q = G^q X_\mathrm{d} \tag{2}$$

where G is mean annual income of the group of people being considered, the exponent q is a function of the fraction of time w spent working ("work–life balance"):

$$q = w/(1-w) \tag{3}$$

and X_d is the discounted life expectancy of the group.[3] It is assumed that G and X_d are independent, although in fact they are interrelated, albeit in complex ways (e.g., higher income could lead to eating better food, prolonging life expectancy, but it could also lead to eating more food, reducing life expectancy). It may safely be assumed that the (commercial) introduction of a nanomaterial leads to an economic benefit (e.g., it becomes cheaper to produce a certain artefact), hence G is increased—if that were not the case the business would soon collapse. With these assumptions we can write (with subscript b denoting benefit):

$$\Delta Q_\mathrm{b} = G_\mathrm{b}^q X_\mathrm{d} - G^q X_\mathrm{d} \tag{4}$$

for the corresponding increase in Q. On the other hand, the risk (estimated from equation 1) can be converted into a reduction in Q, ΔQ_r, due to reduced life expectancy and/or reduced income (e.g., because ill health due to exposure needs to be treated):

$$\Delta Q_\mathrm{r} = G_\mathrm{r}^q X_{\mathrm{d,r}} - G^q X_\mathrm{d} \tag{5}$$

and the technology clearly makes no sense if $|\Delta Q_\mathrm{r}| > \Delta Q_\mathrm{b}$. Depending on the economic structures prevailing, it is possible that the risks and benefits accrue to different groups of people, and it may be worth spending some money (hence reducing G) to mitigate the risk of the technology; the *maximum reasonable cost* of doing so can be estimated using the notion that any mitigating measure will have a certain cost that must be met (via taxation if it is introduced by the government, or by higher prices for the hazardous good if it is introduced by the manufacturer). One can derive the expression [62, 50]

[3] The literature suggests $w \approx 1/8$, implying $q \approx 0.14$.; Thomas et al. [62] found their results to be insensitive over $0.12 \leqslant q \leqslant 0.2$.

$$a = \frac{NG}{q}\frac{\Delta X_{\mathrm{d}}}{X_{\mathrm{d}}}, \tag{6}$$

for maximum reasonable cost a, where N is the size of the population exposed to risk and subjected to mitigating measures.[4]

1.3 *Evaluating the toxicity of nanomaterials*

It is, apparently, very easy to carry out an investigation of some nanomaterial X, especially if it is conveniently available in powder form. One simply sets up an experiment in which large numbers of animals are given increasing doses of X under controlled conditions, and one observes the response. Such experiments are very familiar to the chemical and pharmaceutical industries. Unfortunately, it is as if this very ease of investigation has led to an overwhelming plethora of reported results with nanomaterials, based on experiments that have often been carried out with every appearance of haste, and usually with massive doses far exceeding anything that would be encountered in an occupational or other context. At best, a huge work is now required in order to assess all this data for reliability and consistency. The OECD Working Party on Nanomaterials (WPNM) is attempting to do that, although it is doubtful whether it will be able to keep up with the flood of new results. At worst, much of this data is essentially worthless. An egregious (and, unfortunately, not untypical) example is the study by Poland and nine co-workers entitled "Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathology in a pilot study" [48]. Massive doses of the carbon nanotubes were injected directly into the lungs of the animals, of which there were only four, and only two reacted. The work was widely publicized and appeared to be aimed at promoting the popular perception that carbon nanotubes cause mesothelioma. There are, actually, good theoretical reasons for believing that they can [46] but it has been pointed out that due to its poor quality the work would probably never be admissible as evidence in a court of law [41]; the paper's existence might even (if it were accorded any serious attention) make it harder to establish a causal link between (say) occupational exposure and disease because of the weakness of its apparent underpinning of such a link. A rather comprehensive discussion of the inadequacies of the present state of nanotoxicology—which can be defined as the toxicology of nanomaterials—has been given by Hunt and Riediker [22].

Except in cases of wholesale destruction, such as the devastation caused by an earthquake, or a fire, shelling and bombing in warfare, or deliberate demolition, once nano-objects are incorporated into a matrix (e.g., an organic polymer) their rate of release into the environment generally becomes negligible. Hence, nanostructured materials are, in general, a negligible source of exposure. It might be that the most common source of damage to nanocomposites is presently accidental collisions between motor-cars. If, however, the collision is serious enough for a significant quantity of nanoparticles to be released from automotive nanocomposite components such as bumpers, it is likely that the occupants of the motor-car have suffered other, more serious, bodily injuries. Quantifying the balance of risk in such cases is nontrivial and will

[4] Thomas et al. [63] work out some practical examples (for the inverse problem, whether it is reasonable to spend money on a medicinal drug to combat a natural disease) for the calculation of a. The J-value is the quotient of the actual spend and the maximum reasonable cost a.

not be attempted here. We shall, therefore, confine our attention to exposure to nano-objects: particles, nanotubes, nanoplatelets and the like. Because of their small size, they can readily penetrate into the human body, whereas artefacts made from nanocomposites are usually large and cannot. Unless there has been significant degradation of the matrix (e.g., due to weathering), the nano-objects embedded in it will not normally be released and even contact with the artefact will not usually pose any risk.

Whereas with most of the molecular (chemical) toxins listed in EH40 [10] and similar compilations it can be assumed that if the substance is present in the ambient air it will be taken up by the body, in the case of nano-objects the relationship between presence and uptake is rarely so simple. In fact, the WELs attempt to capture the complexities of what we can call penetrability in a rudimentary way by specifying different exposure limits depending on the particle size; for example, in EH40 silica has three entries, "amorphous inhalable dust and respirable dust", "respirable crystalline", and "fused respirable dust" with successively decreasing limits (by about an order of magnitude each time). It may be more useful to use an elaborated form of equation (1) that explicitly takes account of penetrability:

$$\text{Risk (from the substance)} = \text{Exposure (to the substance)} \times \text{Penetrability} \times \text{Hazard (from the substance)}. \quad (7)$$

The penetrability (§4) could be quantified as the probability that a nano-object arriving at the boundary of the organism is taken up by it in a biologically active form. If one suspects that collective effects affect penetrability (e.g., a group of nano-objects arriving roughly simultaneously within a small area can penetrate more readily than particles arriving in isolation both temporally and spatially) then convolution rather than simple multiplication may, again, be a more appropriate way of combining the factors on the right-hand side.

This paper continues as follows. After briefly reviewing the description of nano-objects (§2), exposure is considered (§3) in both occupational nonoccupational situations. Penetrability and clearance are considered in (§4). Then, the different hazards associated with nanomaterials entering the human body are described (§5). In the final sections, indirect effects of nanomaterials on humans are considered (via their effects on ecosystems on which human life depends, §7) and how the output of the fledgling nano industry compares with natural exposure to nanomaterials (§8).

2. Characteristic features of nano-objects

A nano-object could be unambiguously specified as a list of its atoms giving their identities and positions. Perhaps in the future that is how they will be specified, but for the present it is impracticable for all but the smallest clusters, hence a somewhat coarse-grained description is usually adopted.

Geometry. The type of object is determined by the number of dimensions in the nanoscale: 1, 2 or 3 [23] (fractal dimensions are not considered in this scheme), named nanoplatelet,[5]

[5] The cited technical specification actually names such objects nanoplates, and the two larger dimensions could be of essentially infinite extent. Since for the rest of this article we are only concerned with discrete nano-objects, we shall refer to nanoplates with the two larger dimensions, while not in the nanoscale, nevertheless being small, as nanoplatelets.

nanofibre (further subdivided into tubes (hollow), rods (rigid) and wires (conducting)) and nanoparticle.

All of the object's dimensions should be specified. A rod 3 nm in diameter and 70 nm long would, apparently, be called a nanoparticle despite having an aspect ratio of over 20, since its lengths in all three orthogonal directions are below 100 nm. If both shape and dimensions are specified, there should be no ambiguity. A particle does not have to be spherical; far more elaborate shapes have been synthesized, often called nanoflowers (e.g., [42]), which defy a standardized description.

Most production processes do not produce batches of nano-objects in which all have identical size (and other parameters). The dimensions are distributed and the distribution should be considered part of the specification. If the objects are of irregular shape, there may also be a distribution of shape; which in some cases can be quantitatively characterized from the dimensions (e.g., the shape distribution of ellipsoidal nanoparticles is the distribution of aspect ratio).

Atomic arrangement. The crystal structure (or lack of it) should be specified. If particles have been made by a "top–down" method, it is often assumed that they have the same crystal structure as the bulk material, but this should be confirmed or otherwise by measurements on the particles themselves, for example by selected area electron diffraction. Anomalous atomic arrangements with no bulk equivalent are known to exist in nanoparticles [49].

Chemical composition. Even in the simplest case of a nano-object constituted from a single element, the reactivity of the atoms depends on geometry. Considering nanocubes as an example, atoms on the edges are more reactive than atoms on the plane, and the corner atoms are more reactive still. The situation is considerably more complicated in the case of a binary compound MX. If it is crystalline, depending on the indices of the faces, their chemical constitutions may be very different (all M or all X or equal numbers of both types of atoms). Nano-objects made from compounds are likely to be nonstoicheiometric (i.e., berthollides rather than daltonides). Atoms of the surface cannot fully bond with their partners-in-compound (the carbon nano-objects graphene, nanotubes and the fullerenes are exceptions) and nano-objects have a very high proportion of surface to bulk atoms. The unsatisfied chemical bonding capabilities of the surface atoms are usually satisfied either by scavenging other atoms or molecules from the environment or by a deliberate coating of the nanoparticle with some other compound, often in order to increase stability with respect to agglomeration. A particularly extreme example is the protein "corona"[6] that forms when blood, which is very rich in proteins, comes into contact with a foreign surface [31]. These surface-associated proteins are likely to become denatured [13], making them foreign in the sight of the immune system, which therefore promptly marks them for elimination. Especially in view of the fact that the substances bound to the surface are likely to constitute an appreciable proportion of the total mass of the nano-object, they need to be included in its description. If these substances are macromolecules, conformational information should also be given.

The complexity of the chemical composition increases further in the case of ternary and more elaborate compounds (including substances in which X is a multi-atom entity such as an

[6] Defined as proteins adsorbed to a nonbiological particle and/or sufficiently strongly attracted to it to move together with the particle [25].

oxy anion), and in the case of objects with deliberately engineered internal structure, such as a "core-shell" particle with chemically different core and shell.

Agglomeration. Nano-objects are often produced in agglomerated form,[2] either deliberately as a final step in production or because the balance of surface forces makes them agglomerate. When the medium in which the objects are dispersed changes, the agglomerate may also change. Typically agglomerates are less harmful than dispersed primary particles.

All of these features will influence penetrability and hazard (e.g., [17]); it cannot be asserted that there is a generic kind of nanoparticle that can be used to assess the risk from exposure.

3. Exposure

Apart from direct injection into the bloodstream, inhalation (of an aerosol) is by far the most effective route of entry of foreign substances into the body. We shall, therefore, neglect the other two routes usually considered, dermal and oral. Assuming that the duration of contact with the aerosol is well defined, the main metrology problem is, therefore, the determination of the number of nano-objects per unit volume of the ambient air.[7] The most common approach is to collect the objects by causing them to pass over or through an adhesive or entangling surface under defined conditions for a defined interval and then subsequently analysing the surface [8, 39]; online sensors capable of real time continuous monitoring are also available [8, 39].

3.1 *Occupational exposure (during nanofacture)*

Clearly the highest exposure to artificially produced (engineered) nano-objects is likely to be in the factories producing them.[8] At the same time they will have the resources to minimize exposure by filtering air and providing breathing masks for staff to wear, and to monitor the presence of nano-objects in the air [30].

Production, whether by comminution (some form of grinding or milling) or by nucleation and growth, generally takes place in closed vessels. Accidents apart, the risk of exposure is clearly greatest when the nano-objects are being transferred, perhaps for post-synthesis modification or simply for packing. Nanoparticles in particular are especially fugitive: simply opening a container of them in the air will cause large numbers to escape. Therefore, if the particles can be agglomerated prior to post-synthesis handling,[2] they will be much easier to handle. When they arrive at the end user, they can be dispersed back into the primary particles by the application of modest amounts of external energy.

3.2 *Public exposure (during use)*

Again excluding accidents (such as traffic incidents causing shipping containers containing nano-objects to burst and release their contents) members of the general public are unlikely to be inadvertently exposed to nano-objects.

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[7] The number (or number of moles) of nano-objects is more useful and relevant than the total mass of exposure (note that the molecular weight of a nanoparticle may well be considerably smaller than that of a typical blood protein). Knowing the mean size and shape, one can quickly estimate the corresponding number of surface atoms.

[8] Through his work on diseases contracted by miners [21], including what one would nowadays call silicosis, Paracelsus probably ranks as the founder of occupational nanotoxicology.

Intentional exposure is another matter: they may well apply nanoparticle-containing sunscreen or other cosmetic creams onto their skin, use nanoparticle-containing toothpaste, etc. Dermal and oral routes are likely to be the main means of entry into the human body. It has also been shown that nanoparticles in consumer products tend to group together (agglomerate) [32]; this also tends to happen with aerosols; it offers a safeguard since the general public is unlikely to be exposed to freshly dispersed nanoparticles.

4. Penetrability and clearance

Penetrability has been introduced above, in equation (7), as "the probability that a nano-object arriving at the boundary of the organism is taken up by it in a biologically active form". "Is taken up in a biologically active form" could be construed to mean "taken up by the target organ", which could even be a cell or an organelle within a cell. Penetrability corresponds to the adsorption and distribution phases that precede drug metabolism, part of the ADME framework used in pharmacokinetics. The last phase of ADME is excretion, which corresponds to what is usually called clearance of a nano-object from the body. It can be thought of as negative penetrability and subsumed into the same parameter, which gives the net availability of the nano-object at the site of action.

Penetration is generally a multistep process. We have assumed that it begins by inhalation; see [2] for details of the pulmonary interactions of nano-objects. From the lungs they can readily pass to the bloodstream and thence into individual cells. The epithelium of the lung (the primary organ) is exposed to the highest concentration; the exposure suffered by secondary organs may be two or more orders of magnitude lower. "Pinocytosis is the primary process by which an exogenous entity enters the cell, goes to the specific organelle of the cell and there manifests its cellular function" [38, 26]. Phagocytosis is the process of engulfment of foreign objects by an immune cell [19], and should therefore be considered as part of the clearance process (cf. [3]), which ends with excretion.

There is uncertainty over whether nano-objects can pass into the brain. The blood–brain barrier may be robust but nerve cells may offer channels for conduction [43].

The dependence of penetrability on nano-object characteristics (§2, especially size) is stronger than that of exposure [35, 28]. As a general rule, the smaller the objects, the easier they can penetrate the various barriers such as the epithelium and the endothelium (whence the general suspicion concerning nano-objects [58]); at the same time, the easier they can be phagocytosed and eliminated. This implies that there is an intermediate, doubtless shape-dependent [16],[9] size where the net penetrability is maximal.

5. Hazard

Nano-objects that have penetrated inside the human body can subsequently act in a number of ways [53], which we can conveniently group. The first group comprises what might be called (bio)physicochemical effects: phenomena that do not depend on what might be called living responses, and which could be readily observed *in vitro* in the absence of living cells. The second group comprises effects consequent on the dissolution of the nano-object, following

[9] The interactions of nanoparticles have long been known to be shape-dependent [44].

which the nanoparticulate origin of the dissolved substances is of no significance and the effects are covered by classical toxicology [36]; examples would be nanoparticles of strychnine, potassium cyanide or cadmium selenide. The third group comprises effects only observable within a living organism. These may in turn be divided into single-cell or subcellular responses and responses involving one of the complex subsystems inside the body, notably the immune system.

Group 1: (bio)physicochemical effects

1. in the lungs, interaction with a pulmonary surfactant [12];
2. in the bloodstream, formation of a corona constituted from blood proteins [61];
3. at cell membranes, formation of pores [56].

Group 2: Classical toxicological effects of poisons

Group 3: Effects on cellular machinery

4. persistent attempted phagocytosis: if two dimensions of a nano-object are smaller than the diameter of a macrophage, and one is much greater (i.e., a fibre), the macrophage will attempt to ingest the object but will be unable to do so (Figure 1). The result is inflammation and the release of reactive oxygen species,[10] which can damage biomolecules;
5. platelet activation leading to thrombus formation [5];
6. suppression of the immune system [40, 67, 51];
7. genotoxicity (DNA damage) [4].

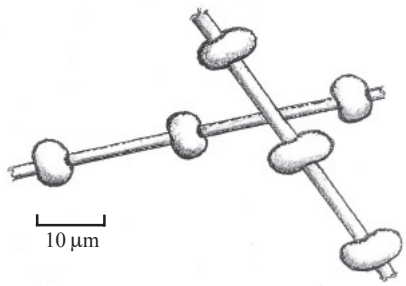

Figure 1. Sketch of a typical scanning electron micrograph of two amphibole asbestos needles within the pleural cavity occupied by several macrophages. Reproduced with permission from [45].

Nano-objects penetrating into the body may serve as carriers for toxins that they have picked up on their surfaces while in the external environment. For example, a poisonous vapour might be present in the workplace, but below the WEL. If nanoparticles are also present in the atmosphere, the vapour may condense on them and thereby be transported into the lungs, yielding an effective exposure well in excess of the WEL. There is a wider scenario of multiple effects, such as simultaneous exposure to different nanoparticles, that has barely been investigated at all. Furthermore, exposure to purportedly a single kind of nanoparticle may actually be exposure to a mixture if the preparation contains a variety of sizes and shapes. If the preparation is merely characterized by its mean size and other features, this variety may be unsuspected. It is important to be aware that whereas exposure is only weakly dependent on nano-object characteristics (§2) the hazards are strongly dependent. The chemical constitution

[10] Certain nanoparticles can generate reactive oxygen species themselves (see, e.g., [34]).

of the surface of the objects is, obviously, expected to be of particular importance since it constitutes the "nano" side of the nano/bio interface.[11]

Broadly speaking, Group 1 will influence Group 3, which in turn will influence higher level physiological functions, such as lung performance [60]. In summary of the above list, it should be emphasized that it only gives a very incomplete picture of the situation. In 2006 Revell wrote "relatively little is known of the biological consequences of exposure to nanoparticles" [53]; seven years later little appeared to have changed since Treuel et al. commented that "the fundamental interactions of nanomaterials with biomatter remain incompletely understood" [65].

6. Variability of individual response

It is salutary to remember that, just as individuals vary enormously in their response to medicinal drugs, they may also show differentiated responses to nano-objects. We still know too little about the detailed pathways of ADME for nano-objects in order to be able to pinpoint particular features of, say, genetic constitution as responsible for hypersensitivity to a particular nano-object, for example (cf. §1.1). The NOAEL approach is supposed to ensure that no individual is subjected to adverse conditions, but we do not even know how variable the response of a population is. Even individual cells show a variable response [27]! Consideration of these aspects also leads to caution in accepting toxicological tests carried out on nonhuman species for determining NOAELs or other input into WELs.

7. Risks to vital ecosystems

If risk over the entire lifecycle of a nanomaterial is to be considered, then we must inquire into the fate of the artefact in which it is incorporated after the use for which it was fabricated has ceased. In comparison with the large volume of data that has been accumulated on toxic effects in humans (and surrogate laboratory mammals), very little has been done to investigate the effects of nanoparticles on other organisms. Deleterious effects of particular nanoparticles have been observed in zebrafish [15], *Daphnia* [37] and even bacteria [7] (apart from intentional bactericidal activity), to give just a few examples. It has also been found that plants are able to take up nanoparticles [14]. The soil in particular is a very complex, still poorly understood ecosystem [54], full of microorganisms, and there is plenty of potential for unexpected effects of unnatural nanoparticles to occur. In the face of such ignorance, caution is advised. Recently it has become popular to impregnate socks and other garments with nanoscale silver particles in order to inhibit the growth of bacteria. These particles are fairly fugitive and liable to be released in significant numbers when the textile is laundered. Given the rather trivial nature of the application, it should be deprecated since the risks to vital ecosystems appear to outweigh the benefits to the quality of human life.

8. "Natural" exposure to nanoparticles

Small particles have been present in the air long before mankind appeared on Earth, chiefly in the form of wind-borne mineral dusts, organic aerosols from forests and carbon from fires.

[11] Lewicka et al. [32] remind us that nanoparticles incorporated into a product may have surface characteristics considerably different from those of the nanomaterial prior to its incorporation.

Civilization has enormously increased exposure. People are increasingly concentrated in cities, where their particle-generating activities are also concentrated, sometimes with dire results (Figure 2). Many of these activities are related to combustion, including the internal combustion engine powering motor vehicles and other engines [29] and pyrometallurgy. The mean particle size of smokes from combustion (including recreational tobacco smoking [57]) tends to be in the micrometre range, with only the tail of the distribution falling below 100 nm (similarly with the products of milling, such as flour). Given that the control of fire for cooking and heating apparently long predates the emergence of *H. sapiens* [55], humans are presumably adapted to protect themselves (to some extent) against deleterious effects of fine particle exposure. Many natural waters, which until relatively recently were often drunk untreated, contain nanoparticles in abundance (see, e.g., [64]). At any rate, the health effects of airborne particulate matter have been well studied [9]. Nevertheless, the products of the nano industry will, increasingly, be smaller objects of compositions not previously encountered, and the question then arises, how rapidly can humans adapt to cope with them (possibly faster than one might think [18])?

Figure 2. Photograph of a street scene in Harbin.

9. Summary and conclusions

The greatest health risk from discrete nano-objects is an occupational one during their manufacture. Since they are subsequently incorporated into other products (including nanocomposites), from which it is hard for them to escape, once they are in normal use exposure to the nano-objects becomes negligible. Only after the other products have been discarded might they be released into the environment, where indirect effects on ecosystems supporting human life could be of greater significance than direct exposure to humans.

Risk can be decomposed into the product (or convolution) of exposure, penetrability to target organs or cells (with clearance considered as negative penetrability), and the actual hazard. If the particle is insoluble, once inside the body it can undergo one of three fates: clearance, apparently innocuous residence inside a cell, and the target of continuous attack by the immune system. The last of these is particularly dangerous. Continuous inflammation ensues, which is associated with reactive oxygen species and, hence, oxidative stress and, in the

longer term, mesothelioma. The most effective triggers of such attack are acicular particles. Soluble particles may be toxic by virtue of the products of their dissolution; the toxicity of insoluble particles will be essentially determined by their surface atoms and molecules.

Although our understanding of the toxicity of nano-objects is still rather incomplete, there is sufficient knowledge available to direct sensible precautions being taken in the factory producing them. It is important to keep in mind that there is no generic toxicity of nanoparticles. Paracelsus' dictum "Alle Dinge sind Gift, und nichts ohne Gift, allein die Dosis macht, dass ein Ding kein Gift ist" [20] is generally applicable, but beyond that the toxicity of a nano-object at a particular dose will depend on its physicochemical characteristics (and, very probably, its history prior to arrival at the target organ).

Published nano-object toxicity work needs to be critically appraised. Up until now there have been too many poorly conceived investigations, with absurdly large doses being given to generate acute toxic effects. Much less attention has been given to the chronic effects of low-level exposures, of both pure preparations of nano-objects and their mixtures.

It is highly important for industrialists whose businesses are based on nanotechnology to master the concepts associated with the toxicity of nano-objects. Legislation is often based on subjective perceptions of risk and, where there is a large divergence between them and reality, robust and realistic demonstrations of reasonable risk are essential if innovation is not to be stifled.

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