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# Why is the (nano-)dose question so important?

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## Summary

Paracelsus postulated that every substance is toxic and that only the dose makes the poison. The question is how to define a certain "dose" for nanomaterials and for nanoparticles, respectively. Why is it impossible to calculate a distinct dose for nanoparticles? The problem is that nanoparticles are very diverse and heterogeneous regarding their chemical and physical properties. It seems rather unlikely that uniform units of measures or parameters characterizing these properties and reflecting the biological effectiveness could be developed. However, the dose calculation for nanoparticles is of high relevance, especially for risk assessment, limit values regulation and recommendations, respectively. Therefore this dossier outlines the correlation between exposition, dose and dose response and it explains why the knowledge of these crucial points is essential and where the gaps in knowledge still remain.

*Paracelsus (1493-1541) by T. Apiryon,*
*© 1995 Ordo Templi Orientis.*
http://hermetic.com/sabazius/paracelsus.htm

## Introduction

A substance might potentially be harmful or even toxic for a biological system, provided that the quantity or the concentration (the "dose") is high enough. The toxic effect (toxicity) increases in case of an increased exposition (or dose). This phenomenon has been known since Paracelsus (1493-1541), the father of toxicology defined: *"All substances are poisons, and there is none which is not a poison; only the dose permits something not to be poisonous."* Even water might be toxic, provided in sufficiently large quantities within a short time. The effect depends on whether a single dose is strong enough (acute dose) or whether low doses are administered over an extended period of time (chronic dose). This principle forms the basis for health standards which determine the maximum permissible concentration of contaminations, for example in food, water or in the environment. Dose calculation is of high relevance for risk assessment as well as for regulations, for instance to determine the maximum allowable concentration (MAC) of chemicals and particles or to determine other limits which do not cause health problems. In the case of nanomaterials, especially for nanoparticles there is to date, no limit regulation or any other regulation referring to dose, because the definition of dose for nanoparticles does not exist. The wherefores will be explained in this dossier.

## What actually is the dose?

The term "dose" nowadays generally refers to a well-defined amount of a chemical substance (or in pharmacology, a drug) causing a certain effect. In radiology the dose stands for a defined quantum of ionizing radiation, for example X-ray or γ-ray. In the field of radiology the dose is calculated by applying dosimetry. Accordingly there are low, strong, high, or lethal doses. The pharmacologic dose is defined as dosage. There are different possibilities to define doses regarding chemical substances [see box].

## Dose-response relationship

To determine a dose-response relationship different doses of a certain substance are experimentally examined regarding their effects. Therefore the identified effects were arithmetically correlated with the particular amount of the substance that was administered. Thus the concentration triggering a distinct effect (in pharmacology) or the degree of the toxicity can be determined. In this way, the functional relationship between the dose of a substance and its effect on an organism can be specified.

There are well known dose-response curves for many chemicals which clearly demonstrate the significant effects of particular amounts of a substance. All chemicals are poisonous if their dose is high enough

**Different ways to define a dose:**

- mass/weight of a dissolved substance per volume (concentration/gram per litre),
- molar concentration of a dissolved amount of substance (number of atoms, to be calculated by the specific weight) per volume (molarity, mol per litre)
  or
- particle density or particle concentration per volume (particle counts per volume)

whereas a low dose has no significant effect at all. Thus the toxicity of a substance is defined by the dose which induces a specific response in a certain biological system. However, there is not always a straight linear dose-response relationship, i.e. a half dose not necessarily results in a half effect. Every chemical is characterized by its own dose-response relationship.

It is assumed that a toxic dose (TD) of a substance takes effect on all individuals (respectively on the analysed objects), but not all individuals react similarly. For this reason $TD_{10}$ or $TD_{50}$ is determined, respectively, at which 10 % or 50 % of the individuals suffer damage from a toxic effect. Another accepted indication is the lethal dose (LD) or $LD_{50}$, respectively. The latter represents a dose at which 50 % of subjects will die. Based on this principle both the European Chemicals Agency (ECHA) and national competent agencies regulate the approved amounts of chemicals and their limiting values.

## Toxicological studies

Toxicological studies examine the dose-response relationship and its effects on biological systems or organisms such as cells and animals. The dose is determined accordingly to the nature oft the toxic substance. There are different concepts of toxicity [see box].

Dose and dose-response relationship are the main toxicological concepts anyway. However, toxicity is also a function of the effective biological dose. The latter depends on the amount of the particular substance received over a specific period by the target organ. Toxicological studies also assay whether and how the substance can enter the target organ within the body (toxicokinetics). The route by which a dose is exposed to is an important parameter regarding the dose, because different exposure routes can cause different dose-response effects depending on whether they are taken transdermally, inhaled, ingested, or injected. The dose-response relationships also depend on the period of time over which the dose was received.

The exposure time can be acute, sub-chronic, or chronic. Accordingly, the toxicity can also be acute, sub-chronic or chronic. However, exposition and toxicity are not per se affiliated with each another, because an acute exposition does not necessarily result in an acute toxicity. For example, a unique high concentration of a chemical substance can be health-impairing even years later. Moreover, other factors such as gender, age, body weight, medication etc. are able to affect the effect in the target organ.

## Toxicology and Nanomaterials

Particles with dimensions on the nanoscale are not really new. Such particles (for example carbon black or the family of the silicates, $SiO_2$) are used in larger quantities for a long time in different areas. The nano-dimension of these particles develops rather at random than on purpose. On the other hand it is possible to generate specifically so called homodisperse nanoparticles (they are about the same size) with modified and/or structured surfaces. Considering the remaining ambiguity about the relevance of configuration, surface properties, electric charge, coating etc., for toxicity is currently only a little or nothing known about the relation between certain particles and their potential biological behaviour. However, it is known that industrial or natural combustion release side-products such as the so called ultrafine particles, ultrafine suspended particulate matter, or ultrafine aerosols. They involve heterodisperse particles (of different sizes) which are typically in the size range of 100 nanometers. The effects of these particulates are mostly known whereby specific chemical or shape-induced effects (fibre principle) occur; long-term exposure of granular dusts (biopersistence) could significantly contribute to inflammations. However, the mechanisms of homodisperse as well as of surface-modified nanoparticles are unknown. There is not even evidence how to define a dose. There are many reasons for surface reactivity as measure for the dose, but how should it be calculated?

Thus, the fundamental principles of nanotoxicology are understood, however specific information on the definition of dose and on the determination of dose-response effects are frequently lacking. Because the number of nanomaterials to be examined for toxicological effects increases very rapidly, their toxicological examination presents a considerable challenge[1] or even seems unrealistic. To enable dose estimation and to conduct experiments with realistic doses it is necessary to determine suitable units for measuring doses[2-4].

However, it is known that some nanoparticles are able to cause relevant toxic effects. These effects are related to their physical and chemical properties (for example size/surface, catalytic property, etc.)[5]. The toxicity of nanoparticles can be larger than their bulk material, because the surface area to volume ratio of nanoscale materials is far bigger. In addition, some nanomaterials contain metals or other substances which can alter their toxic properties. Because of the extreme heterogeneity of the chemical and physical properties of nanoparticles, it is very unlikely that one single measure or one single unit is sufficient for dose calculation. At present, surface, number and size of the particles are usually indicated. The question is whether these data are sufficient, in order to finally define meaningful and practicable values for legislation and standardisation. In addition to surface reactivity, shape and biopersistence, the accumulation in certain organs or cells should be taken into account. The question remains therefore what effects should be determined in order to identify the surface reactivity. Different so called *biological endpoints* are conceivable, for example the formation of free radicals or the viability of certain cells in cell culture.

Concepts of toxicity

- The intrinsic **chemical-driven toxicology** considers the effect of soluble substances, atoms, or molecules which interact with biological systems, whereby the mass serves as a metrical quantity/dose (molarity or concentration per unit).
- The **morphology-driven toxicology** considers fibre-like substances like asbestos particles or fibre-like zeolite etc, whereby the number of fibres per unit serves as a metrical quantity/dose.
- The **radiation-driven toxicity** considers the radiation energy applying the deposited energy as a dose.
- In the field of **nanotoxicology** it has been suggested to apply the surface reactivity as the most important parameter. The area and the surface reactivity are considered to be the most important quantities in terms of dose. However, it is still unclear whether these parameters are actually the most important.

## So what is the problem?

Exposition and dose of nanoparticles differ in that exposition refers to the possible amount of free nanoparticles (or area or number of atoms, respectively), whereas the term "dose" reflects on uptake, retention, and biological effectiveness of a specific nanoparticle. Hence the following information is essential for dose calculation:

- Exposition: not the external (from the outside), but the internal exposition (the amount of substance absorbed; see above) is relevant
- A measuring unit considering chemical and/or physical properties (surface, reactivity, etc.)
- Specifications relating to routes of exposure (dermal, inhalation, gastrointestinal, injection)
- Information on toxic effects (biological effects)
- Relevant details regarding dose-response relationship
- Information relating on exposure times (acute, sub-chronic, or chronic)
- Information on specific enrichment in single organs, tissues, and cells
- Data on cell-, tissue-, and organ-specific effects
- Specifications relating biopersistence
- Further information regarding the respective nanoparticles.

Currently, there is little information available delivering all these necessary data. Unfortunately, there is no dosimetric concept that would allow a more or less uniform dose calculation. Due to the heterogeneity of nanoparticles and because of knowledge gaps of its reciprocal effects with living systems a standardised calculation (algorithm) is currently unrealistic.

### Notes and References

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### Conclusions

Thus, do nanomaterials question the paradigm of toxicology "only the dose makes the poison" (Paracelsus)? Or only does the dose have to be correctly defined in order to be able to answer the question with "no"? The issue still remains unanswered.

First of all, dose calculation for nanoparticles would be important for risk assessment, for definition of limit values and for recommendations, respectively.

Due to missing data, parameters for dose calculation of nanoparticles cannot be defined. In order to fill the knowledge gaps by systematic studies, and in order to develop a realistic dose concept for nanoparticles, there is still an urgent research need.

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