Approaches for establishing human health no effect levels for engineered nanomaterials

K Aschberger and F M Christensen
Nanobiosciences Unit, Institute for Health and Consumer Protection, European Commission-JRC, 21020 Ispra, Italy

E-mail: Karin.aschberger@ec.europa.eu

Abstract. Current Technical Guidance Documents for preparing risk assessments, like the guidance for the implementation of REACH, have limited focus on chemical substances in the particulate form and generally do not focus on substances in the nanoform. Within the ENRHES project a comprehensive and critical scientific review of publicly available health and safety information on four types of nanoparticles was performed. Based on the identified exposure and hazard data, basic human health risk assessment appraisals were carried out for fullerenes, carbon nanotubes, nano-silver and nano-titanium dioxide. These risk assessment appraisals followed the structure of a regulatory risk assessment and if possible and relevant, it was attempted to derive indicative human no-effect levels from key studies by applying assessment factors as suggested in the technical guidance document for REACH. These assessment factors address differences and uncertainty related to exposure features between test animals and humans (time, respiratory volume), other interspecies and intraspecies differences and factors for extrapolation to chronic duration. If required, the severity of effects and the quality of the database can be addressed by additional factors. Recently other procedures for deriving human no-effect levels have been published and these are compared to the ENRHES basic risk assessment appraisals. The main differences were observed in relation to evaluating the differences in animal and human exposure situations and interspecies differences, and in applying assessment factors for intraspecies differences for local effects. The applicability of the REACH guidance for nanomaterials is currently being investigated for possible adaptations, considering the specific behaviour and mode of action of nanomaterials.

1. Introduction
In the framework of the European Regulation REACH, registrants (importers and manufacturers) have to demonstrate that risks are controlled for manufacturing and use of their substances. This involves, where possible, derivation of DNELs (derived no-effect levels) or DMELs (derived minimal effect levels for non-threshold effects) to be compared to workplace exposures and/or exposures of the general public (consumer exposure and exposure via the environment). Risks are considered properly controlled when exposures are lower than the DNEL.

1 The opinions expressed in this publication are those of the authors and not necessarily those of the European Commission.
The provisions of REACH apply to nanomaterials [1] and therefore the guidance for preparing a REACH chemical safety assessment [2] in principle also covers engineered nanomaterials, however it currently includes very little reference to substances in particulate form and generally do not focus on substances in the nanoform.

Within the ENRHES project (Engineered Nanoparticles: Review of Health and Environmental Safety; http://nmi.jrc.ec.europa.eu/project/ENRHES.htm) and follow up activities, basic risk assessments for four types of nanomaterials were performed. For these basic risk assessment appraisals, indicative human no-effect levels were determined based on the REACH guidance (chapter R.8) [2] for certain types of carbon nanotubes, fullerenes, nano-silver and nano-titanium dioxide (for details see Aschberger et al. [8] Christensen et al. [9]). These papers identified several challenges with deriving no-effect levels based on current methodology. Other and sometimes more sophisticated approaches for risk assessment of nanomaterials have lately appeared in the public domain. In the current manuscript, different proposed procedures for deriving human no effect levels are compared and discussed.

2. Establishing human no-effect levels.

Human exposure limit values like the DNEL or occupational exposure limits (OEL) are generally based on dose descriptors (No (lowest) observed adverse effect level/concentration N(L)OAEL/N(L)OAEC or benchmark dose) for critical effects from animal toxicity studies which then have to be modified to human exposure situations by employing assessment factors to account for differences (extrapolation) and uncertainty.

When establishing human no-effect levels, the following criteria have to be taken into account:

- Exposure route
- Key study (relevance, reliability, nanomaterial characterization)
- Toxicokinetics
- Nature and severity of effect:
  - threshold or non-threshold mechanism,
  - local – systemic effect
- Dose descriptor
- Modification to the starting point
- Assessment factors

Inhalation is expected to be the most relevant exposure route for nanoparticle exposure, especially at the workplace due to the possibilities of particles becoming airborne. So far, internationally harmonised toxicity testing procedures for nanomaterials are not available. The preferred study type for a risk assessment is an inhalation study for a sub-chronic or chronic duration. However it is technically very difficult to produce and maintain an appropriate and representative atmosphere of nanoscale particles and these studies are therefore complex and expensive and not very often performed. Further, for inhalation studies, test substances may be technically manipulated to increase the dustiness and this may not represent the real human exposure situation. Therefore hazards identified in rodents inhalation toxicity studies need to be carefully interpreted when used for establishing human no effect levels.

3. Examples for deriving human no-effect levels, following the REACH guidance

In the following we present an approach for deriving human no-effect levels for inhalation by applying the default assessment factors for local effects as described in the REACH guidance [2]. Default assessment factors should be used in the absence of substance specific or analogous data allowing the establishment of appropriate values. The presented evaluation was made based on the available information in the open literature and includes many uncertainties, e.g. related to nanomaterial characterisation. Therefore it was necessary to rely on default assessment factors and the results should not be used for any regulatory purposes or decision making. Consequently, to de-couple from
regulatory purposes, we preferred to use the term INEL (Indicative No-effect Level) instead of DNEL. This methodology is compared to other approaches reported in the literature and the results are discussed.

In this manuscript we mainly focus on chronic occupational inhalation exposure to carbon nanotubes (CNTs) and nano-TiO$_2$, where most information for attempting derivation of no-effect-levels is available. The selected key studies are rat inhalation studies of high reliability and sufficient nanomaterials characterisation, where the animals were exposed for 6 hours per day and 5 days per week. For multiple walled carbon nanotubes (MWCNT) we selected 2 recent 13-week inhalation studies (OECD 413) in compliance with good laboratory practice with Nanocyl 7000 (5-15 nm x 100-1000 nm, 90% purity) [3] and Baytubes® (10 nm x 200~1000 nm; 99.1% purity) [4]. The latter included a post-exposure period for up to 6 months. The LOAEC for Nanocyl was reported as 0.1 mg/m$^3$, while the same concentration was reported as a NOAEC for Baytubes®. For nano-TiO$_2$ (21 nm particles) we selected a 13-week inhalation study (including assessment of pulmonary responses up to 52 weeks post-exposure) [5] with a NOAEC of 0.5 mg/m$^3$.

The observed effects in these toxicity studies like local inflammation and granulomas in the lung suggested a threshold mechanism and it seemed therefore relevant to derive human Indicative No-Effect Levels. However there is uncertainty with regard to possible genotoxic and/or carcinogenic effects of these nanomaterials and therefore in principle a non-threshold effect cannot be generally excluded (for further details please refer to Johnston et al. [6,7], Aschberger et al. [8] and Christensen et al. [9]). Under the assumption of a threshold mechanism of toxicity, modifications to the starting point and assessment factors were applied to derive INELs for the selected substances.

3.1. Modifications to the starting point
The exposure duration of the animal in the experiment (usually 6h) is corrected for the assumed daily worker exposure (usually 8h). Workers at light activity have a slightly higher breathing rate and respiratory volume (10 m$^3$) as compared to rest (6.7 m$^3$). The modifications for these conditions (6/8h*6.7/10 m$^3$) correspond to a factor of 2 for workers.

3.2. Assessment factors
Assessment factors are numerical values used to address differences between experimental data and the human situations taking into account the uncertainties in the extrapolation procedure and the available data set.

3.2.1. Interspecies differences. The interspecies assessment factors, to extrapolate effects in one species (e.g. rats) to other species (e.g. humans), address possible differences in the mechanisms influencing dose and toxicity of chemicals. It might be that these factors need to be adapted for nanosized materials considering the nanoparticle specific properties, as the behavior in the respiratory tract (deposition, persistence) and the possible different nature of toxic effects. There are different approaches for interspecies factors and some approaches split the interspecies factor into toxicokinetics and toxicodynamics, whereas in the REACH guidance they are split into allometric scaling (depending on the tested animal species) and remaining interspecies factors (ECHA 2008).

In the selected studies the observed effects were local and did not depend on metabolic rate or systemic absorption and therefore allometric scaling is not applicable. Thus, only the factor for remaining interspecies differences (default 2.5) was applied and in the case of nano-TiO$_2$ it was suggested to be reduced to 1.5, as it has been shown that rats were more sensitive than mice and hamsters [5].

For decisions on the extent of interspecies differences it is important to understand the nature of the observed effect. In the case of CNTs, it was postulated that the critical mechanism triggering pulmonary inflammation is dependent on the volumetric overload of alveolar macrophages [4,10]. "Lung overload", is a phenomenon more extensively seen in rats due to different deposition and clearance capacities. Symptoms caused by lung overload are not expected to be manifested to the same
extend in larger mammals such as dogs, monkeys and humans [11] and therefore the rat should be considered to be more susceptible than humans for these kind of effects [12].

3.2.2. Intraspecies differences. For intraspecies variation default assessment factors of 5 and 10 are recommended in the REACH guidance to account for interindividual differences in workers and the general population, respectively. A factor of >1 for workers is considered justified as some workers may have a predisposition to react more sensitive to particle exposure, e.g. asthma. However some other approaches [10,13,14] (see below) consider a factor of 1 sufficient.

3.2.3. Exposure duration. The assessment factors for duration can take into account the extrapolation of the duration from sub-acute (default 6) or sub-chronic (default 2) to chronic exposure, unless it is shown, that exposure duration is not relevant for the manifestation or severity of effects. In the basic risk assessment appraisal we applied the default factor of 2 for sub-chronic to chronic extrapolation. It is not known how the observed effects would develop under prolonged exposure and therefore no statement on the appropriateness of the default factor can be made. The effects could become more severe, but there could also be recovery. The extrapolation factor for the duration could change both ways if more information on the progression of the effects became available and this would thus have an impact on the results and conclusion of the risk assessment. In the case of MWCNT Baytubes® [4], where the sub-chronic study was extended by a post-exposure period of 6 months with no progression of the effects it might be justified to reduce this assessment factor.

3.2.4. Dose-response. If in the experiment no concentration without an effect has been determined, an assessment factor (default factor of 3) has to take account for the extrapolation from the Lowest Observed Adverse Effect Concentration (LOAEC) to a No Adverse Effect Concentration (NAEC). This factor can be changed, depending of the severity of the effects. In the inhalation study with MWCNT Nanocyl [3] the lowest tested dose of 0.1 mg/m$^3$ showed subclinical findings (minimal granulomatous-type inflammation in the lung and lung-associated lymph nodes) and therefore we suggested a reduced assessment factor of 2 for extrapolation from LOAEC to NAEC.

The severity of the effect can be taken into account by assessment factors, however in none of the exercises it was considered appropriate. There has also been a debate concerning the appropriateness of using nano-specific uncertainty factors to account for unknowns associated with conducting risk assessments in this field compared to the experiences with ‘conventional’ chemicals. However this was not applied in the basic ENRHES RA appraisals.

Further assessment factor can be applied to account for the quality of whole database when data are scarce or poor in quality. The overall assessment factor should be balanced in using the different factors to avoid accounting for uncertainties in multiple ways, so the whole approach would become over-precautionary.

The overall assessment factors applied to the dose descriptors (including the modification to the starting point) were 50, 100 and 30 and the calculated INELs for the investigated ENMs were 2 μg, 1 μg and 17 μg for MWCNT (Baytubes), MWCNT (Nanocyl) and nano-TiO$_2$ respectively.

4. Analyses of different approaches for deriving exposure limit values

The following other approaches for deriving human no effect levels have recently become available: Pauluhn [10], Lecloux and Luizi [15], NIOSH [16] and the Japanese risk assessment within the NEDO project [13,14]. They partly refer to the same substances and toxicity studies as considered in the ENRHES risk assessment appraisals.

1) Pauluhn [10] has recently published an attempt to derive an occupational exposure level (OEL) specifically for multi-walled CNT as produced by Bayer, called Baytubes®. On the basis of a repeated
(subchronic) rat inhalation studies, a mechanistic (conceptual) model was developed forming the basis for interspecies extrapolation leading to an overall extrapolation factor of 2 and an OEL value of 0.05 mg/m$^3$. No further factors for intraspecies differences or duration were applied.

The interspecies differences account for differences in alveolar deposition, ventilation and the time dependent particle accumulation reflecting the known species-specific differences in particle clearance half-times in rats and humans. The first step of the calculation relates to the differences in deposited dose between animals and humans. In the author's view the dosimetrics adjustment factor does not call for any deposition related dosimetrics adjustment between rats and humans (i.e. 1) because the higher respirability of particles by humans is averaged out by the increased ventilation of rats.

A comparison of the number and volumes of alveolar macrophages (AM) between rats and humans suggests that humans are six-times more resistant to attaining lung overload conditions, which are assumed to be a trigger for the observed toxic effects. Other differences addressing the lung volume, lung weight, lung surface area and total amount of surfactant [17] may be normalised by the body weight. Combining the body weight differences with number and volumes of alveolar macrophages, leads to an AF of 1.7 ~ 2 [10]. Intraspecies adjustments were not applied due to the lack of any systemic bioavailability of the nano-particles and based on the rationale that this type of portal-of-entry related toxicity (overload) is independent on metabolism. The commonly applied default value for the worker's ventilation (10 m$^3$/working day and adult) was considered to be implicitly overconservative [10].

2) Another risk assessment on a multi-walled CNT produced by Nanocyl for BASF was also based on a 90-day inhalation study in rats [3]. Starting from a LOAEL of 0.1 mg/m$^3$, an assessment factor of 40 was applied, resulting in an estimated “no effect” concentration in air of 2.5 µg/ mg/m$^3$ for 8 h/day exposure [15]. No further details on how this overall assessment factor was derived is presented.

3) Within the NEDO project on "Research and Development of Nanoparticle Characterisation Methods", the Japanese National Institute of Advanced Industrial Science and Technology has conducted risk assessments for 3 types of engineered nanomaterials (ENMs), including CNTs [13] and nano-TiO$_2$ [14]. The adjustments for human exposure situations are based on differences in lung deposition between rats and humans and no further factors were applied. No factor for toxicokinetics was considered necessary, as adverse effects related to the pulmonary inflammation, are local and depend only on the alveolar deposition fraction of particles. Differences of the deposition fraction and surface area of pulmonary alveolus had been already adjusted in the estimation of the provisional NOAELs. For toxicodynamics, they argued that rats have even greater sensitivity to particle exposure than humans.

For CNTs, Kobayashi et al. [13] calculated an acceptable exposure concentration of 0.21 mg/m$^3$ for CNTs from a NOAEC of 0.37 mg/m$^3$ in a 4 week-inhalation study based on CNT deposition on the pulmonary alveoli (= factor 0.85). The deposition into lung was calculated by multiplying the exposure concentration in air with alveolar deposition fraction (depending on particle size), exposure frequency (8h/day x 5 d/week) and breathing rate during working (36 m$^3$/day), divided by bodyweight (60 kg). From the air concentration of 0.37 mg/m$^3$, a lung dose rate was calculated at 6.0 µg/kg/day and further divided by the overall uncertainty factors (UF) of 2, resulting in 3.0 µg/kg/day, which was converted back to 0.21 mg/m$^3$ (time weighted average, 8 h/d, 5 d/w). The UFs cover toxicokinetics (1) and toxicodynamics (1) differences and the extrapolation of exposure period (2, sub-acute to chronic). No factor for intraspecies differences was considered.

For nano-TiO$_2$ [14] the amount deposited on the lungs of the experimental animals was calculated by multiplying the NOAEC of 2 mg/m$^3$ [5], with respiratory minute volume, exposure time and deposition fraction, and finally dividing by the bodyweight. After applying an UF of 2 (for duration: sub-chronic to chronic) the deposited dose was calculated back to an acceptable exposure value for humans of 1.2 mg/m$^3$ (which corresponds to an overall factor of 1.7 compared to the NOAEC).
4) The US National Institute for Occupational Safety and Health (NIOSH) [16] derived a Recommended Exposure Level (REL) for <100 nm TiO$_2$ of 100 $\mu$g/m$^3$ (for worker exposure 10 hours per day, 40 hours per week) aiming at a maximum risk for lung cancer of 1/1000 over a work life, but noted that the risk is possibly lower - perhaps even zero. This REL was derived based on data by Heinrich et al. [18] and using the linearised upper bound on risk from the multistage model. In this study an increased mortality and lung tumours in rats were reported following chronic inhalation of high concentrations of nano-TiO$_2$ (10 mg/m$^3$). Based on that data and a review of the literature NIOSH concluded that nano-TiO$_2$ is carcinogenic to rats and that it cannot be ruled out that nano-TiO$_2$ is also carcinogenic in humans. It is assumed that the mechanism for carcinogenicity would not be chemical specific, but caused by chronic inflammation following pulmonary overload, and thus caused by the particle nature of TiO$_2$.

5. Consideration on the dose metrics
In the above presented examples, the dose descriptors from the inhalation studies and the derived human no-effect levels were expressed in the mass metric. The mass metric allows a straightforward comparison of external exposure concentrations and retained cumulative doses in the lung for example for CNTs [10]. However, the same mass of nanoparticles has often been shown to be more inflammogenic than the same mass of larger particles of the same chemical composition [5]. It is thus also important to consider the influence of particle size and surface as well.

Therefore, mass metric alone does not always provide the optimal metric for the observed effects, so other metrics, like for example, the number concentrations, the surface area, or the shape (fibrous) can be better metrics for relating dose to the observed effects of a specific nanomaterial. On the other hand, some pragmatism might be needed in relation to selecting proper metrics for doing regulatory risk assessments. In conclusions, the most appropriate (unifying) metrics of dose are still unresolved [19], between others, due to the diversity of different types of engineered nanomaterials.

6. Conclusion
Different approaches for deriving human no-effect levels have been suggested by different groups.

The approach following the REACH guidance by largely applying the suggested default assessment factors usually lead to higher overall assessment factors and lower human no-effect levels as compared to other reported approaches. The higher overall assessment factors result mainly from higher factors for interspecies differences and the application of intraspecies factors for workers.

For interspecies differences other approaches have mostly calculated the differences of deposited nanoparticles doses in the lung of rodents and humans. These differences were around a factor of 2 in the OEL approach for MWCNTs [10] and are in the same range as the factor for modification to the starting point (according to the REACH guidance) taking into account the differences in exposure duration in the toxicity study and the workplace, and the respiratory volumes at rest as compared to light activity. In the Japanese approach within the NEDO project [13,14] however these differences correspond to a factor of 0.85. The additional default factor of 2.5 for remaining interspecies as in the REACH guidance [2] is the main reason for numerical differences of assessment factors in the different approaches. The possibility for reducing this factor to 1 is currently not provided in the guidance for inhalation exposure, but as chemical specific assessment factors (CSAFs) should always be given preference over default assumptions, such a reduction might be justified in certain cases. Some authors suggest that the exposure conditions for laboratory animals to particles are quite different from those for humans and may lead to an overestimation of the human risk [20]. For low-toxic particles the observed effect in rats is often inflammation secondary to lung-overload – to which rats seem to be more sensitive than humans [11,12]. However, no generalisations are possible and the definition of CSAFs requires reliable chemical specific data and good understanding of the fundamentals of the biological response to the material.

Differences were also observed in the application of intraspecies differences. While all of the approaches we have compared with the ENRHES approach considered such a factor not necessary, we
do not share this view and the REACH default factor of 5 or eventually a reduced factor of 3 seems justified to us. The AFs for extrapolation to chronic duration are important, especially as some of the nanoparticle effects will only manifest after chronic exposure. If it can be shown, that effects do not depend on the duration of exposure, these factors can be reduced.

Considering the possibility that properties and mechanisms of nanomaterials may differ fundamentally from the non-nanoscale materials and chemicals, the default assessment factor values should be evaluated for their appropriateness for nanomaterials. There are currently two projects ongoing (REACH Implementation Projects RIP-oN 2 and 3) which aim at developing advice on how the REACH guidance on information requirement and chemical safety assessment could be updated to address the specific properties of nanomaterials.

References
[1] EC (European Commission) 2008. Nanomaterials in REACH. Doc. CA/59/2008 rev.1. 6th Meeting of the REACH Competent Authorities 15-15 December 2008. Brussels
[2] ECHA (European Chemicals Agency) 2008. REACH Guidance on Information Requirements and Chemicals Safety Assessment. http://guidance.echa.europa.eu/guidance_en.htm
[3] Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Merltler M, Wiensch K, Gamer AO, van Ravenzwaay B, Landsiedel R 2009. Inhalation toxicity of multi-walled carbon nanotubes in rats exposed for 3 months Toxicol. Sci. 112 273-5
[4] Pauluhn J 2010a. Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures Toxicol. Sci. 113 226-42
[5] Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI. 2004. Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles Toxicol. Sci. 77 347-57
[6] Johnston HJ, Hutchison GR, Christensen FM, Peters S, Hankin S, Aschberger K, Stone V 2009b. A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes; the contribution of physicochemical characteristics Nanotoxicology 4 207-246
[7] Johnston HJ, Hutchison GR, Christensen FM, Peters S, Hankin S, Stone V 2009a. Identification of the mechanisms that drive the toxicity of TiO2 particulates; the contribution of physicochemical characteristics Part Fibre Toxicol. 6:33
[8] Aschberger K, Johnston HJ, Stone V, Aitken RJ, Tran CL, Hankin SM, Peters SAK, Christensen FM 2010. Carbon nanotubes – assessment of the feasibility and challenges for human health risk assessment based on open literature Crit. Rev. Toxicol. 40 759-90
[9] Christensen FM, Johnston HJ, Stone V, Aitken RJ, Hankin S, Peters S, Aschberger K 2010. Nano titanium–dioxide - feasibility and challenges for human health risk assessment based on open literature Nanotoxicology, Early online. DOI: 10.3109/17435390.2010.504899
[10] Pauluhn J 2010. Multi-walled carbon nanotubes (Baytubes®): Approach for derivation of occupational exposure limit. Regul. Toxicol. Pharmacol. 57 78-89
[11] Born PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdörster E 2006. The potential risks of nanomaterials: a review carried out for ECETOC Part Fibre Toxicol 3:11
[12] Brown JS, Wilson WE, Grant LD 2005. Dosimetric comparison of particle deposition and retention in rats and humans. Inhal. Toxicol. 17 355-85
[13] Kobayashi N, Ogura I, Gamo M, Kishimoto A, Nakaniishi J 2009. Risk Assessment of Manufactured Nanomaterials – Carbon Nanotubes (CNTs) - NEDO project "Research and Development of Nanoparticle Characterizations Methods (P06041)" Interim Report issued October 16, 2009; available at http://www.aist-riss.jp/main/?ml_lang=en
[14] Hanai S, Kobayashi N, Ema M, Ogura I, Gamo M, Nakaniishi J 2009. Risk Assessment of Manufactured Nanomaterials – Titanium Dioxide (TiO2) - NEDO project "Research and
Development of Nanoparticle Characterizations Methods (P06041)" Interim Report issued October 16, 2009; available at http://www.aist-riss.jp/main/?ml_lang=en

[15] Lecloux AJ, Luizi F 2009. The safe use of carbon nanotubes in coating applications. presentation held at the European Commission's 3rd stakeholder dialogue on nanomaterials "Safety for success", 3-4 November 2009 in Brussels; available at: http://ec.europa.eu/health/ph_risk/documents/ev_20091103_co11_en.pdf

[16] NIOSH (National Institute for Occupational Safety and Health) 2005. NIOSH Current Intelligence bulletin: Evaluation of Health Hazard and Recommendations for Occupational Exposure to Titanium Dioxide. DRAFT. NIOSH. Available via: http://www.cdc.gov/niosh/review/public/TI02/pdfs/TIO2Draft.pdf

[17] Oberdörster G, Oberdörster E, Oberdörster J 2007. Concepts of nanoparticle dose metric and response metric. Environ. Health Perspect. 115 A290

[18] Heinrich U, Fuhst R, Rittinghausen S, Creutzenberg O, Bellmann B, Koch W, Levens K 1995. Chronic inhalation exposure of wistar rats and two different strains of mice to diesel engine exhaust, carbon black, and titanium dioxide. Inhal. Toxicol. 7 533-56

[19] Maynard AD 2007. Nanotechnology: the next big thing, or much ado about nothing? Ann. Occup. Hyg. 51 1-12

[20] Oller AR, Oberdörster G 2010. Incorporation of particle size differences between animal studies and human workplace aerosols for deriving exposure limit values. Regul. Toxicol. Pharmacol. 57 181-94